Mathematical Modelling of *Echinococcus multilocularis* Transmission in Foxes in Zurich (Switzerland)

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Dedicated to my two families

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Acknowledgements

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Summary

Human alveolar echinococcosis (AE), caused by *Echinococcus multilocularis*, is a severe emerging or re-emerging zoonotic disease in some parts of the northern hemisphere. In Europe, this parasite has predominantly a wild animal cycle, with red foxes (*Vulpes vulpes*) as definitive hosts and some rodent species as intermediate hosts. The growing presence of foxes in densely populated large cities of Europe, such as Zurich (Switzerland), might bring a concomitant higher environmental contamination of parasitic eggs leading to an increased risk for humans. Through the review, development and application of statistical and mathematical models to parasitic data the present research provides with a better understanding of the epidemiology of this parasite and the performance of some of the currently available tests for diagnosis and thus, might be of assistance in the optimization of the control and prevention programs.

Chapter 2 presents a comprehensive systematic review on significant risk factors for *Echinococcus multilocularis* and *Echinococcus granulosus* infection in animal hosts obtained through the employment of classical statistical methods. The most relevant factors associated with *E. granulosus* infection in dogs (main definitive hosts) included being fed with raw viscera, being stray or free to roam and having owners that showed a lack of knowledge about the disease, which was in turn related to deprived living conditions. Whereas *E. granulosus*' infection in livestock (main intermediate hosts) was primarily linked to the hosts' age and to specific external conditions that can facilitate the survival of parasitic eggs in the environment. *E. multilocularis* transmission dynamics in animal hosts were associated with a complex host population dynamics and predator-prey relationships established between the main definitive hosts (foxes) and the main intermediate hosts (rodents) which are influenced by spatial and climatic changes.

Chapter 3 characterises the spatio-temporal dynamics of *E. multilocularis* force of infection (FOI) in Zurich foxes (measured in parasites insults per unit time experienced by fox). The data gave evidence of a periodic FOI with minimums in

summer (95% confidence interval, *CI*, 0.27-1.27) to maximums in winter (95% CI, 6.87-7.05). In addition, the FOI varied distinctly among urban habitats indicating that foxes from the outside of the city were exposed to a higher number of parasite insults annually raging from 0.7-3.9 to 9.35-9.7 (95% CI) compared to the foxes collected closer to the city center, which raged from 0.1-0.8 to 1.6-2.0 (95% CI). The data did not support the presence of parasite-induced immunity in Zurich foxes.

Chapter 4 quantifies the spatio-temporal differences of *E. multilocularis* infection pressure (IP) in Zurich foxes (measured in parasites that would develop in the fox after infectious insult per unit time). The data supported best the existence of a periodic IP with different amplitudes across urbanization zones, presenting higher peaks during the cold seasons (2,500 parasites/insult/periurban fox and 8,300 parasites insult/urban fox) compared to the warm seasons (100 parasites/insult/periurban fox and 3,000 parasites insult/urban fox). In addition, the data indicated the existence of variations in IP among age groups only in foxes from the periurban zone, although it did not support the presence of parasite-induced immunity.

Chapter 5 applies Bayesian latent class models to the results of four tests employed for the diagnosis of *E. multilocularis* in Swiss foxes. The results from the Bayesian analysis determined the true parasite prevalence in foxes (95% credible interval, *CI*, 43.1-66.4%), suggested that host age and co-infection with other cestodes were significantly associated with parasite infection and estimated the diagnostic test sensitivities and specificities of the diagnostic tests employed. The 95% CI of the sensitivities for the necropsy and the sedimentation and counting technique (SCT), the detection of parasite DNA material from the parasitic eggs found in fox faeces using the polymerase chain reaction (PCR), the polyclonal (pAb) and the monoclonal (mAb) enzyme-linked immunosorbent assays (ELISAs) ranged between 82.7-93.4%, 48.5-61.0%, 48.0-63.9% and 55.3-70.8%, respectively. The 95% CI of the specificities for the egg-PCR, pAb-ELISA and mAb-ELISA were estimated to ranged between 87.3-99.1%, 55.8-75.6% and 60.1-79.4%, respectively. The specificity for the necropsy and SCT was previously assumed to be 100%.

Zusammenfassung

Die humane alveoläre Echinokokkose (AE), verursacht durch *Echinococcus multilocularis*, stellt eine *emerging* oder *re-emerging disease* in Teilen der nördlichen Hemisphäre dar. In Europa herrscht der herrscht der Parasitenzyklus in Wildtieren vor. Der Rotfuchs (*Vulpes vulpes*) ist der Hauptwirt und einige Nagetierspezies fungieren als Zwischenwirte. Die zunehmende Präsenz von Füchsen in dicht besiedelten, grossen europäischen Städten, wie auch in Zürich (Schweiz) geht möglicherweise einher mit einer grösseren Umweltkontamination mit Parasiteneiern, die dann zu einem höheren Risiko für Menschen führt. Mithilfe eines Reviews, sowie der Entwicklung und Anwendung von statistischen und mathematischen Modellen für die Parasiten-Datensätze liefert diese Arbeit ein besseres Verständnis der Epidemiologie dieses Parasiten und der Leistungsfähigkeit einiger aktuell verfügbarer Diagnostiktests. Die Erkenntnisse können für die Optimierung von Kontroll- und Präventionsprogrammen nützlich sein.

Im Kapitel 2 werden die Ergebnisse eines umfassenden systematischen Reviews zu signifikanten Risikofaktoren (basierend auf klassischen statistischen Methoden) für Tier-Infektionen mit *E. multilocularis* und *Echinococcus granulosus* dargestellt. Die wichtigsten Faktoren, die assoziiert waren mit einer *E. granulosus* Infektion in Hunden (definitiver Hauptwirt) beinhalteten Verfütterung von rohen Innereien, Streunen oder Freilauf und Besitzer mit einem mangelndem Wissen zu dieser Krankheit zu haben - was wiederum verknüpft ist mit niedrigem sozio-ökonomischem Status. Bei den Nutztieren hingegen, den wichtigsten Zwischenwirten, war die Infektion mit höherem Wirtsalter und weiteren Faktoren assoziiert, die das Überleben der Parasiteneier in der Umwelt fördern. Transmissionsdynamiken von *E. multilocularis* in Tierwirten war assoziiert mit komplexen Wirtpopulationsdynamiken und Beute-Räuber-Beziehungen zwischen den definitiven Hauptwirten (Füchse) und den Hauptzwischenwirten (Nager), die wiederum beeinflusst sind von räumlichen und klimatischen Veränderungen.

Kapitel 3 charakterisiert die spatio-temporalen Dynamiken der *E. multilocularis* Infektionskraft (gemessen in Parasiteninsulten pro Zeiteinheit per Fuchs). Basierend

auf den Daten gibt es Evidenz für eine Periodizität der Infektionskraft mit einem Minimum im Sommer (0.27-1.27, 95% Konfidenzinterval) und einem Maximum im Winter (6.87-7.05, 95% Konfidenzinterval). Weiterhin variierte die Infektionskraft deutlich zwischen urbanen Habitaten. Dabei waren die Füchse von ausserhalb der Stadt im Vergleich zu Füchsen, die in der Nähe der Stadtmitte gesammelt wurden, einer Exposition mit einer höheren Zahl an Parasiteninsulten mit Werten die von 0.7-3.9 bis 9.35-9.7 bzw. von 0.1-0.8 bis 1.6-2.0 (95% Konfidenzinterval) reichten, ausgesetzt. Die Daten geben keinen Hinweis auf eine parasiten-induzierte Immunität in Zürcher Füchsen.

Kapitel 4 quantifiziert die spatio-temporalen Unterschiede des *E. multilocularis* Infektionsdrucks (gemessen in Parasiten, die sich im Fuchs nach Infektionsinsult pro Zeiteinheit entwickeln). Die Daten unterstützen die Annahme eines periodischen Infektionsdruck mit unterschiedlichen Amplituden innerhalb der Urbanisationszonen. In der kalten Jahreszeit treten dabei mit (2,500 Parasiten/Insult/periurbaner Fuchs und 8,300 Parasiten/Insult/urbaner Fuchs) höhere Peaks auf als in der warmen Jahreszeit mit (100 Parasiten/Insult/periurbaner Fuchs und 3,000 Parasiten/Insult/urbaner Fuchs). Weiterhin wiesen die Daten darauf hin, dass die altersabhängige Variabilität des Infektionsdrucks nur in Füchsen der periurbanen Zone auftrat. Eine parasiten-induzierte Immunität wurde hingegen nicht unterstützt.

Im Kapitel 5 werden Bayesianischen latent Klasse Modelle für die Testergebnisse von vier Diagnostiktests von *E. multilocularis* in Schweizer Füchsen verwendet. Laut Bayesianischer Analyse liegt die wahre Prävalenz in Füchsen zwischen 43.1-66.4% (95% Glaubwürdigkeitsintervall). Die Ergebnisse weisen darauf hin, dass Wirtsalter und Ko-Infektion mit anderen Cestoden signifikant mit Parasiteninfektion assoziiert sind und beinhalten auch die geschätzten diagnostischen Sensitivitäten und Spezifitäten der verwendeten Diagnostiktests. Die Sensitivitäten vom mikroskopischen Nachweis nach Sedimentation- und Zählverfahren im Kot aus den Sektionen, von der Parasitemeier-PCR aus Fuchskot, einem polyklonalen- (pAb) und einem monoklonalen (mAb) ELISA schwankten zwischen 82.7-93.4%, 48.5-61.0%, 48.0-63.9% und 55.3–70.8%. Die geschätzten Spezifitäten der Parasiteneier-PCR, des pAb- und mAb-ELISA lagen bei jeweils 87.3-99.1%, 55.8-75.6% und 60.1-79.4%. Es

wurde die Annahme getroffen, dass die Spezifität des mikroskopischen Nachweises 100% ist.

Introduction

‣ Echinococcosis overview

Parasite taxonomy and life cycle

Echinococcus spp. are small endoparasites from the class Cestoda (tapeworms) and family Taeniidae, that can infect many different species of animals, including humans. In the current state of the on-going efforts to understand its phylogenetic, nine species are recognised: *Echinococcus granulosus* sensu lato (s.l.) complex (split into five species), *Echinococcus multilocularis*, *Echinococcus shiquicus, Echinococcus oligarthra* and *Echinococcus vogeli* [1-4]. These tapeworms have an indirect life cycle that involves a definitive host and an intermediate host. The adult tapeworm is carried in the small intestines of the definitive hosts, which are carnivores mostly members of the Canidae family, where they grow by generating cestode segments (proglottids) from the scolex. The parasitic eggs or gravid proglottids are produced via sexual reproduction and shed into the environment through the faeces. Afterwards, the intermediate host, which includes a wide range of herbivorous and omnivorous mammalians, gets perorally infected through the uptake of the tapeworm eggs. After ingestion, the eggs hatch and develop into the larval stage of the parasite (metacestode). The metacestode, a fluid-filled cyst that will contain the protoscoleces, migrates and establishes in the body tissues. The parasite's life cycle is completed when the intermediate host or part of its tissue containing a metacestode is ingested by the definitive host.

Human echinococcosis

Humans can become aberrant intermediate hosts following the accidental ingestion of parasitic eggs. Transmission to humans may happen through direct contact with he definitive host or, indirectly, from the contaminated environment. After ingestion, the metacestodes can affect various organs causing a severe zoonosis called human echinococcosis. Among the nine species currently recognised, the larvae of some of the species belonging to the complex *Echinococcus granulosus* s.l. and the larvae of *Echinococcus multilocularis* are especially relevant as they can cause the two main clinical forms of human echinococcosis: cystic echinococcosis and alveolar

echinococcosis. Both conditions have a difficult diagnosis and an expensive and complicated treatment that requires a long-term commitment and a holistic approach to be tackled [5]. Furthermore, rural communities in close contact with their domestic animals and areas with reduced levels of health education and health services are particularly vulnerable to these diseases [6]. Globally, they are believed to cause a median estimate of 206,000 cases each year and to be responsible for a median estimate burden of 871,000 disability-adjusted life-years (DALYs) [7,8]. Therefore, human echinococcosis remains a major public health problem in the most intensely endemic areas and, in addition, it is also an emerging or re-emerging disease in some other parts of the world [9]. The World Health Organization (WHO) has included human echinococcosis within the group of neglected tropical diseases and in the list of priority neglected zoonotic diseases for which WHO supports concerted control efforts [10,11].

‣ Cystic echinococcosis

Etiology

Cystic echinococcosis (CE), with a worldwide geographical distribution, is caused by the larvae of some species belonging to the complex *Echinococcus granulosus sensu lato* [9]. *E. granulosus* s.l. encompasses the following five different species: *E. granulosus* sensu stricto (s.s.) (or *E. granulosus* strains G1-G3), *E. equinus* (or *E. granulosus* strain G4), *E. ortlepi* (or *E. granulosus* strain G5), *E. canadensis* (or *E. granulosus* strains G6/G7, G8 and G10) and *E. felidis* [12-15). The status of the genotype G9 is still uncertain [16]. *E. granulosus* s.s. (G1-G3) is the one most frequently associated with human CE [13]. Recent research suggests that *E. canadensis* (G6/G7) might have been an underestimated cause of human CE [15], whereas the genotypes G8 and G10 of *E. canadensis*, with a more limited distribution, are far less common [13]. Conversely, just isolated cases have been related to *E. ortlepi* whereas *E. equinus* appears not to be zoonotic [13]. Finally, *E. felidis* is unknown to cause human infections [13]. *E. granulosus* s.s. (G1) along with *E. canadensis* (G6/G7) have an estimated contribution of 88.44% and 11.07%, respectively, to the CE infections worldwide [13].

E. canadensis (G6/G7), commonly named camel and pig strains, are mainly supported by the synanthropic cycle with domestic dogs as definitive hosts and a wide range of domestic ungulates as intermediate hosts (i.e. camel, pig, cattle, reindeer, goat, sheep), although *E. canadensis* (G6) has been reported in wolves [17]. The G6 genotype has been found in human cases from America, Asia and Africa, while the G7 genotype appears to be mostly restricted to some parts of central and Eastern Europe [18]. *E. granulosus* s.s. transmission is essentially maintained by a synanthropic cycle involving domestic dogs as definitive hosts and sheep and other farm ungulates (goats, cattle, buffaloes, camels, etc.) as intermediate hosts.

E. granulosus senso stricto epidemiology

E. granulosus s.s. (G1) is the most widespread genotype posing the greatest threat to human health, especially in rural communities where there is a close contact between the humans and their livestock and working dogs [19-21]. Farm dogs have often access to disposed offal from the home slaughtering of livestock [22], resulting in a direct risk to its owners or anyone coming in direct contact with them. Despite high human incidence rates are frequently linked to livestock rearing areas, the underlying socio-economic determinants are key factors that can promote the direct or indirect parasite transmission between dogs and humans [23,24]. Risk factors related with poor sanitation, poor living standards or low income have been suggested to play a significant role to human infection [19,20,23,24]. Therefore, CE falls particularly heavily on the poor and marginalized populations in regions with limited resources, where it meets the criteria for a neglected disease [25]. Human infection with *E. granulosus* s.s. leads to the development of *Echinococcus* cysts (metacestode) that is most frequently localised in the liver and the lung, although in can virtually involve any organ and structure [26]. Clinical symptoms, if any, occur after a long incubation period that can last several years and they vary depending on the localisation, number and size of the cyst(s). CE is a chronic disease that is not easy to be diagnosed without the proper medical resources [6]. Besides, the disease prognosis can be fatal if left untreated.

Public health importance

CE shows the highest prevalence and socioeconomic burden among the different forms of human echinococcosis [7]. In spite of the lack of existing data on CE occurrence, the median estimate of cases is 188,000 (95% Uncertainty intervals, *UI*, 157,000-1,770,000) every year worldwide [8]. In hyper-endemic parts of Peru, Argentina, East Africa, Central Asia, and China, prevalence levels can reach as high as 5-10% [27]. Likewise, the hydatid infection is a frequently finding at slaughterhouse level with ovine reported prevalences varying from less than 10% to 83% in some parts of Peru, Brazil, East Africa, China, Central Asia and countries from the Mediterranean basin [28]. The median estimate of the global burden related to CE is of approximately 184,000 DALYs per annum (95% UI, 88,100-1.59 million DALYs) [8]. In addition, there is also a global financial burden derived from livestock production losses estimated to be around $2 billion per annum [29]. In spite of its severity, CE is a preventable disease as human behaviour plays a critical role in the perpetuation of the domestic cycle. Some of the suggested approaches for the control and prevention of CE entitle the regular deworming of dogs, education campaigns, adequate slaughter and post-mortem inspection, livestock vaccination and effective surveillance in animal and human population [27].

‣ Alveolar echinococcosis

Etiology

Alveolar echinococcosis (AE), less common than human CE but lethal if untreated, is caused by the metacestode stage of *E. multilocularis*. The adult stage of *E. multilocularis* inhabits the small intestine of wild carnivores, mostly foxes (genera *Vulpes* and *Alopex*) and other wild canids such as wolves, coyotes and raccoon dogs that act as definitive hosts [26]. Eggs can remain viable in the environment for up to 1 year given the suitable moistly and low temperature conditions exist [30]. Small mammal species, mainly rodents members of the family Arvicolidae and Cricetidae, act as the natural intermediate hosts after the ingestion of the parasitic eggs shed in the faeces of the definitive hosts [26]. The parasite cycle is completed when the fox or another suitable carnivore predates on a rodent infected with cysts. Domestic dogs,

and to a lesser extent cats, play an important role in some endemic areas where a semi-domestic life cycle occurs [19,31,32].

E. multilocularis epidemiology

E. multilocularis is generally considered to be restricted to the northern hemisphere, particularly affecting regions of China, the Russian Federation and countries in continental Europe and North America [9]. Humans become accidental intermediate hosts through the oral intake of the parasitic eggs found in contaminated food, water or in their own hands after handling an infected definitive host [33]. The *E. multilocularis'* life cycle involve foxes, pets, rodents and a suitable ecosystem that maintains host populations and parasitic eggs in the environment. Therefore, some of the frequently reported risk factors for AE involve the contact with potential definitive hosts (mainly foxes and dogs), contact with contaminated fomites (water, soil, plants, food, etc.) and having an agricultural occupation [19,31,34]. In addition, the presence of particular regional landscapes and weather conditions can also enhance parasite transmission by providing a suitable habitat for the susceptible intermediate host species or by facilitating the egg survival in the environment [30,35]. A history of dog ownership and contact with dogs have been significantly associated with risk of human AE disease in the China foci [36,37]. In the human host the metacestode stage develops in to a multilocular structure with many small cysts that is predominantly located in the liver, although it can infiltrate other organs and tissues. This tumour-like proliferation can remain asymptomatic for several years, being a progressive and chronic disease with a frequently fatal outcome if untreated [26].

Public health importance

E. multilocularis represents a major problem in certain rural communities of central and western China, including the Tibetan plateau, bearing over 90% of the global burden of AE [38]. Outside the endemic areas of China, AE is generally rare and sporadic with a median estimate of cases occurring worldwide of 18,000 (95% UI, 11,000-30,000) every year [8]. The global median estimated burden related to AE of 688,000 DALYs per annum (95% UI, 409,000-1.1 million DALYs) is greater than the one for CE due to the high case fatality ratio of AE [8]. Prevention and control of

AE can be quite challenging as the life cycle of *E. multilocularis* is mainly supported by wild animal species, which are difficult to target [39]. Nevertheless, human echinococcosis is at least partially preventable and may be highly cost effective to control if long-term measures are put in place [40]. In order to design effective intervention strategies, a sound understanding of the epidemiology of this disease is essential. Humans may get infected from animal hosts but the parasite cannot be directly transmitted between humans, being a cyclozoonosis. Therefore, successful control of human AE depends on interruption of parasite transmission and reduction of human exposure through the targeting of animal hosts [41]. Suggested control measures encompass the regular deworming of wild and domestic definitive hosts, the management of wild and stray host populations, the delivery of health educational campaigns aiming to reduce the individual infection risk and the development of parasite transmission models to describe and predict the parasite situation in a cost-effective manner [42].

‣ Epidemiological situation for *E. multilocularis* in Europe

E. multilocularis in humans

AE, originally restricted to a well-defined endemic area in Central Europe, can currently be found at the north, west and east of the continent [9]. The historic core endemic areas for AE have been localised in Germany, Switzerland, Austria and France but the number of human cases reported in the Baltic region indicates that the disease seems to be emerging fast in other regions [43]. Despite of AE be considered a rare disease it poses a substantial threat to the population at risk in the main endemic areas [44]. In 2015, 135 confirmed cases of AE were notified in the EU supposing a 64.6% increase compared to 2014 [45]. However, the real epidemiological situation in Europe is believed to be more serious due to the underreporting/misdiagnosed cases and the long latency period that characterises this disease. In the European context, reported risk factors for AE include living in rural settings, having an agricultural occupation or performing garden-related activities (i.e. famers, gardeners, collecting wood, having a kitchen garden) [34,44]. In addition, having a dog, cat ownership or to

participate in hunting activities have been also identified as to be associated with AE [34,46].

E. multilocularis in animals

The red fox (*Vulpes vulpes*) is still considered the main definitive host in Europe [39]. Other wild carnivores, such as the Artic fox, the raccoon dog, the golden jackal or the wolf, might also play a role in parasite transmission [47]. Red foxes from central and north-eastern Europe show the highest parasite prevalences of over 10% [47]. Only the United Kingdom, Finland, Ireland, Malta and Norway (except for the Arctic Archipelago of Svalbard) have confirmed absence of *E. multilocularis* [48]. The delayed increasing and emerging of AE in Europe could be grossly linked to the rise of the fox populations following the success of the rabies vaccination programme [49], their expansion and adaptation to the urban areas [50] and the reported increase of *E. multilocularis* prevalence in foxes [51-53]. These factors facilitate the intensification of the environmental contamination with infective parasite eggs especially in settings where the human and fox presence intersects, such as the peri-urban areas [39,54,55]. Foxes colonise human settlements attracted by the abundance and accessibility of anthropogenic food sources [56] deriving in high densities of animals co-habitating with humans [57]. Nevertheless, lower parasite prevalences have been frequently reported in foxes found in the centre of the cities compared to rural settings [58-61] and there has been no evidence of an increasing of human cases within the cities [44]. Infection rates might be lower because of the reduce availability of intermediate hosts for *E. multilocularis* in the city centre due to the fragmentation or lack of suitable habitats for rodents [59,60]. However, transition areas between rural and urban settings that provide a suitable habitat for arvicolids species, the main intermediate hosts for *E. multilocularis* in Europe, and where high fox population densities concur, can be hot-spots for the infection of human and their pets [55,59,62]. As dogs have the potential to substantially contribute to the environmental contamination of parasitic eggs and also have a close contact to humans, they should also be considered as a likely source of infection and, thus, be taken into account when designing parasite control programs [40,63].

The sharply variations in parasite presence reported within a very short distance point out the complexity of the local ecology processes behind *E. multilocularis* dynamics [64]. Some of the external elements controlling parasite transmission include the anthropogenic factor (i.e. urbanisation of the landscape, human attitude towards foxes, etc.) and the environmental factors that affect parasite egg survival shaping the type of habitat and resources that, ultimately, determine hosts' densities and their predator-prey relationship [39,65]. In addition, individual features such as host age, host gender or the existence of potential parasite-induced immunity mechanisms are also proposed examples of internal determinants for *E. multilocularis* infection between animal hosts [58,66,67]. Furthermore, the distribution of the *E. multilocularis* biomass in the red fox it has been reported to be highly aggregated [58,68] resulting in few individuals harbouring the majority of worms. Therefore, information on parasite abundance, not only infection status, appears to be of critical importance when estimating human risk of infection [69]. Such complexities have fuelled the use of mathematical models to study *Echinococcus* transmission in a very cost-effective way.

‣ Modelling *E. multilocularis* transmission

Study data

Information on parasite prevalence and abundance of *E. multilocularis* in foxes collected within the municipality of Zurich, previously gathered in the context of the Integrated Fox Project, was kindly offered by the Institute of Parasitology of the University of Zurich to be modelled as part of the present research. This data set, formerly described in [58,59], was employed to evaluate some hypothesis on the spatio-temporal variations in the force of infection (parasite insults per fox per year) and the infection pressure (number of parasites established per parasite insult per fox per year) in foxes in Zurich (Paper 2 and 3 of the present thesis).

In addition, as part of the European Research Programme on Emerging and Major Infectious Diseases of Livestock (EU-Project EMIDA), the identification of *E. multilocularis* in foxes collected in the midlands of Switzerland was performed using four different diagnostic techniques: the sedimentation and counting technique (SCT),

the detection of antigens in faeces using two different enzyme-linked immunosorbent assays (ELISAs) and the identification of parasite DNA isolated from eggs found in the faecal content using the polymerase chain reaction (PCR) technique. This data set was employed to fit latent class models using a Bayesian approach to estimate the true disease prevalence, the transmission parameters and the diagnostic tests' characteristics (Paper 4 of the present thesis).

Laboratory work

There are several diagnostic alternatives to identify *E. multilocularis* in the definitive host. However, these methods do not have the ability to discriminate with 100% accuracy between infected and not infected foxes. By not acknowledging the absence of a gold standard test the outcome of many epidemiological field studies, and the conclusions drawn from them, might render unreliable. One of the proposed solutions to overcome this problem is through the adoption of latent class models. These models recognise the lack of a perfectly accurate diagnostic test and integrate the results of the available imperfect tests as different information sources all related to the unknown true status. In the present research three types of diagnostic tests were performed on foxes. Below there is a brief description of the performing of these three types of diagnostic tests at the Institute of Parasitology of the University of Zurich:

Necropsy followed by the sedimentation and counting technique (SCT)

The small intestines of the foxes were removed, cut in three pieces, opened and placed in a container with 0.9% of physiological saline solution. Then, the intestinal mucosa was scraped manually and introduced into a container with saline solution and left for 15 minutes so the content could sediment. After discarding the supernatant, the containers were refilled with more saline solution. After repeating this process at least four more times it was obtained sediment ready for visual examination with a stereomicroscope. The adult stages of *E. multilocularis* were then identified based on their morphological characteristics.

Copro-antigen enzyme-linked immunosorbent assay (ELISA)

During the necropsy, two grams of faecal samples were retrieved from the rectum of the fox intestines. The faecal material was mixed in a vortex with 6 ml. of phosphate

buffer saline (PBS)-Tween inside a tube and left a 4°C overnight. Next, the tube is mixed again and centrifuged for 10 min at 1600 g at 4°C. Next, the supernatant was analysed using two copro-tests specific for *E. multilocularis* diagnosis and produced at the Institute of Parasitology of Zurich: the polyclonal ELISA (pAb-ELISA) using rabbit and chicken egg antibodies [70] and a recent elaborated monoclonal ELISA (mAb-ELISA) using rat and rabbit antibodies (unpublished).

Copro-DNA detection by polymerase chain reaction (PCR)

The sediment obtained from the faecal samples taken from the rectum after removing the supernatant for the copro-test analysis was used for DNA isolation. For sample preparation it was followed the procedure described by [71] based on the combination of sieving and flotation in zinc chloride solution to concentrate the parasitic eggs. All samples were examined by microscope for *Taeniid* eggs. Samples with *Taeniid* eggs or doubtful were confirmed using a multiplex egg-PCR. The DNA isolation from the parasitic eggs and the subsequent performance of the multiplex egg-PCR was done following [72].

Mathematical models

Mathematical models provide a logical and low-cost tool for studying *E. multilocularis* epidemiology by representing parasite transmission dynamics using mathematical equations. Epidemiological parameters that cannot be measured by mere observation, such as infection pressure or immunity rates, can be estimated through the employment of standard mathematical techniques of integration or by computer simulations [73]. Adopting models that account for stochasticity in the values of their parameters enables the inclusion of variability and uncertainty in their estimates [74]. This allows the adoption of suitable probability distributions that best describe the over-dispersed distribution of worms in the foxes. Furthermore, methods that consider the lack of accuracy inherent to the results from imperfect diagnostic tests can be incorporated to the model building so that the true parasite prevalence and the unknown diagnostic test characteristics can be estimated from the observed data [75]. Moreover, the flexibility of the modelling approach even allows for the incorporation of ecologic aspects related to parasite transmission, such as seasonality or other

spatio-temporal factors [76,77]. Therefore, the modelling of *E. multilocularis* offers a cost-effective opportunity to gain a better insight on the parasite dynamics using field data while accounting for the mentioned complexities so meaningful conclusions can be drawn from the results.

E. multilocularis **transmission models**

Numerous models can be found in the literature to describe *Echinococcus* transmission [78]. For the present research, age-stratified SIR models (Susceptible, Infected and Removed] initially developed by Roberts et al. [79,80] and modified by Torgerson et al. [81], were selected as previous data on parasite distribution in Swiss foxes showed a link between host age and parasite infection and worm burdens [58,82,83]. Moreover, these models also account for the possibility of parasite-induced immunity allowing the testing of this hypothesis on the study data [84]. Although the models were originally produce to describe *E. granulosus* dynamics they can be easily adapted to *E. multilocularis* as it has been previously done [73]. As the topic of this research focus on *E. multilocularis* transmission in foxes, the model building concentrated on parasite prevalence and abundance only in the definitive host, the red fox. These models stratify the red fox population into compartments that represent their infection and immune status, if applicable, at a particular age. The transition between compartments is described by ordinary differential equations (ODE), which derivatives measure the rates of change of hosts in reference to infection status or parasite loads in the population at time or host age t. These equations were modified according to the research questions wanted to be addressed.

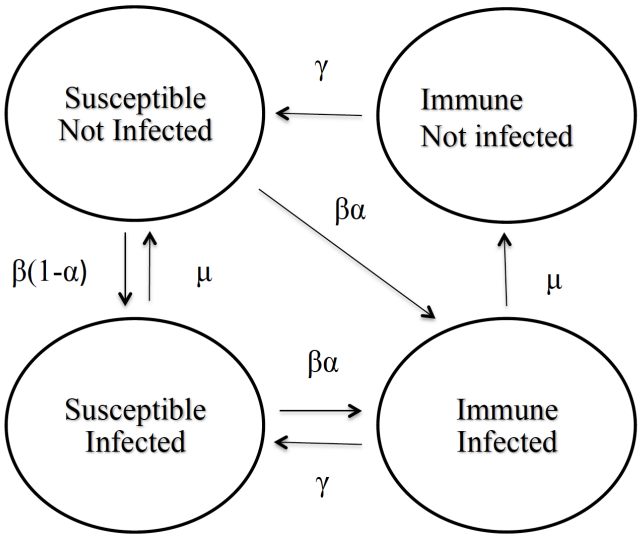

Figure 1. Graphical representation of the *E.multilocularis* transmission in foxes.

For a constant population, the definitive host can be classified in four compartmental states representing the proportion of foxes, at certain age, which are susceptible and not infected, susceptible and infected, immune and infected or immune and not infected. Transitions rates between states are described by the following parameters: β as the force of infection, μ as the parasite death rate, α as the probability of immunity on exposure and γ as the rate of immunity loss.

Diagnostic uncertainty

There are several diagnostic alternatives to identify *E. multilocularis* in the definitive host. However, these methods do not have the ability to discriminate with 100% accuracy between infected and not infected foxes. By not acknowledging the absence of a gold standard test the outcome of many epidemiological field studies, and the conclusions drawn from them, might be unreliable. One of the proposed solutions to overcome this problem is through the adoption of latent class models. These models recognise the lack of a perfectly accurate diagnostic test and integrate the results of the available imperfect tests as different information sources all related to the unknown true status.

‣ Scope and Structure of the Present Research

The scope of this research is to broaden the knowledge of the epidemiology of *E. multilocularis* in Switzerland through the estimation of the values of its transmission parameters and the formulation and testing of hypothesis. To accomplish this objective, real field data on parasite infection and worm loads in foxes was fitted to transmission dynamics models tailored to answer specific questions on the epidemiology of *E. multilocularis*.

Main Research Questions

- What are the risk factors for *Echinococcus* infection in animal hosts that have been determined through the employment of associative models?
- What is the yearly rate at which susceptible foxes are challenged with *Echinococcus* infection within the municipality of Zurich? Does it vary across seasons and habitat types? Is the hypothesis of existing parasite-induced immunity supported by the data?
- What is the annual number of parasites that developed in foxes after being challenged with *Echinococcus* infection within the municipality of Zurich? Are there any differences across seasons and habitat types? Is there an age-dependent distribution of worms? Is the hypothesis of existing parasite-induced immunity supported by the data?
- What is the true *E. multilocularis* prevalence in foxes from the midland regions of Switzerland after accounting for the uncertainty related to the currently available diagnostic tests? What are the characteristics of these tests on the study population? Can we determine any associations between infection status and the information gathered on potential risk factors? Is there any difference in the establishment of the optimal cut-off for the ELISA test if a more empirical method is used instead of considering the necropsy and the SCT as the gold standard test?

Thesis Papers

In order to answer these research questions four main studies were undertaken: a systematic review on risk factors for parasite infection in animal hosts, the mathematical modelling of parasite prevalence, the mathematical modelling of parasite abundance and the employment of laboratory diagnostic methods for parasite detection in field samples to use these data for the evaluation of their diagnostic performance through the application of Bayesian latent class models.

1. A systematic review of the epidemiology of Echinococcosis in domestic and wild animals

The review offers a comprehensive insight into *Echinococcus* epidemiology providing with a compilation of existing knowledge on the significant risk factors for echinococcosis infection in animal hosts. The results of this review provide this research with an informed basis for the developing of parasite prevalence and abundance models as it offers a better understanding of the processes behind *Echinococcus* transmission while highlighting remaining aspects in need to be further investigated. A second stage to assess the mathematical models applied to Echinococcosis was planned, but was abandoned due to the publication by a different research group [78].

2. Dynamics of the force of infection: insights from *Echinococcus multilocularis* infection in foxes

Information on parasite prevalence in red foxes collected in the city of Zurich (Switzerland) was modelled in order to characterise quantitatively the force of infection (measured in parasite insults that the definitive host is exposed to per unit of time) of *E. mulitlocularis* among seasons and different urban types and to explore the possibility of presence of parasite-induced immunity.

3. Mathematical modelling of *Echinococcus multilocularis* abundance in foxes in Zurich, Switzerland

Information on parasite abundance of red foxes collected in the city of Zurich (Switzerland) was modelled in order to characterise quantitatively the infection

pressure (measured in number of parasites per year harboured by the definitive host per unit of time) of *E. mulitlocularis* among seasons and different urban types and to explore the possibility of presence of parasite-induced immunity.

4. Latent class models for *Echinococcus multilocularis* diagnosis in foxes in Switzerland in the absence of a gold standard

The mathematical models developed assume that the infection status of a fox is known with certainty. In reality, this is not true and in order to further develop the transmission models the diagnostic sensitivity and specificity of tests used to assess infection in foxes should be evaluated. Once known, these can be used in a future step to introduce stochasticity of the data when fitting to models. This idea was part of the work of the Swiss National Science Foundation project that supported this research. In order to achieve this, information on parasite infection status of foxes through the employment of four diagnostic tests was gathered and fitted to Bayesian latent class models to determine their performance, the possible existence of test dependencies, the true parasite prevalence and the potential association of parasite infection and some risk factors. In addition, the threshold values of the ELISAs were determined through an empirical method to assess whether it had an impact on test results or not.

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Paper 1

A Systematic Review of the Epidemiology of Echinococcosis in Domestic and Wild Animals

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PLOS | NEGLECTED TROPICAL DISEASES

A Systematic Review of the Epidemiology of Echinococcosis in Domestic and Wild Animals

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Abstract

Background: Human echinococcosis is a neglected zoonosis caused by parasites of the genus *Echinococcus*. The most frequent clinical forms of echinococcosis, cystic echinococcosis (CE) and alveolar echinococcosis (AE), are responsible for a substantial health and economic burden, particularly to low-income societies. Quantitative epidemiology can provide important information to improve the understanding of parasite transmission and hence is an important part of efforts to control this disease. The purpose of this review is to give an insight on factors associated with echinococcosis in animal hosts by summarising significant results reported from epidemiological studies identified through a systematic search.

Methodology and Principal Findings: The systematic search was conducted mainly in electronic databases but a few additional records were obtained from other sources. Retrieved entries were examined in order to identify available peer-reviewed epidemiological studies that found significant risk factors for infection using associative statistical methods. One hundred studies met the eligibility criteria and were suitable for data extraction. Epidemiological factors associated with increased risk of *E. granulosus* infection in dogs included feeding with raw viscera, possibility of scavenging dead animals, lack of anthelmintic treatment and owners' poor health education and indicators of poverty. Key factors associated with *E. granulosus* infection in intermediate hosts were related to the hosts' age and the intensity of environmental contamination with parasite eggs. *E. multilocularis* transmission dynamics in animal hosts depended on the interaction of several ecological factors, such as hosts' population densities, host-prey interactions, landscape characteristics, climate conditions and human-related activities.

Conclusions/Significance: Results derived from epidemiological studies provide a better understanding of the behavioural, biological and ecological factors involved in the transmission of this parasite and hence can aid in the design of more effective control strategies.

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Introduction

Echinococcosis is a zoonotic parasitic infection caused by the larval stage of several species belonging to the genus *Echinococcus*. Human echinococcosis results following the direct or indirect infection from canid hosts, which are themselves infected by various domestic and wild mammals. *Echinococcus spp.* are found throughout the world, although some species have restrictive distributions. Echinococcosis is a major public health concern, particularly in developing regions with limited economic resources. Furthermore, there are indications of an increasing number of cases in certain areas so it is becoming an emerging or re-emerging disease [1–4].

This article will focus on *E. granulosus* and *E. multilocularis*, as these are responsible for virtually all the human and animal burden of the disease. *E. granulosus* is now recognised as having a number of genotypes and molecular evidence suggests there may be a number of species. Hence, *E. granulosus* genotypes 1–10 are now commonly referred to as *E. granulosus sensu stricto* (genotypes G1–G3), *E. equinus* (G4), *E. ortleppi* (G5) and *E. canadensis* (G6–G10) [5–7]. Additionally, mitochondrial studies have identified *E. felidis* as a distinct species although phylogenetically closely related with *E. granulosus sensu stricto* [8]. Of these, *E. granulosus* sensu stricto, *E. ortleppi* and *E. canadensis* cause human cystic echinococcosis (CE) whilst *E. multilocularis* causes alveolar echinococcosis *(AE)*. *E. equinus* is not believed to be zoonotic and the pathogenicity of *E. felidis* to man is unknown.

CE is usually maintained by the domestic cycle (dog/domestic ungulate) and represents a persistent zoonosis in rural livestock-raising areas where humans cohabit with dogs fed on raw livestock offal [9]. AE is mainly supported by a sylvatic cycle (fox/rodents), which can be linked with domestic dogs and cats [10]. AE is confined to the northern hemisphere, representing a major endemic disease in the western and northwestern parts of China [11]. High infection rates have also been reported for domestic dogs in China [12,13], where they are likely to play a significant role in human infection [14,15]. It is also an emerging disease in central Europe coinciding with the growth of fox populations and their expansion towards the urban areas [1]. Although AE is less common than CE it poses a major threat to human health since it is more difficult and costly to treat.

Author Summary

Echinococcosis is considered a neglected zoonotic disease caused by the larval form of *Echinococcus spp*. tapeworms. Humans become infected through the accidental intake of parasitic eggs excreted by the faeces of definitive hosts (dogs, foxes and other canids). Infection involves the development of cysts, primarily in the lungs and liver, causing damage as they enlarge like a slowly growing tumor. Transmission is facilitated by the general lack of awareness of infection factors and epidemiological models can identify them. Nevertheless, there has never been a systematic review summarizing the significant determinants for echinococcosis in animals. One hundred publications were included in the results after evaluating 1,935 entries and screening the references lists of the eligible papers. Principal factors associated with canine infection included the access of dogs to infected offal, allowing dogs to roam free, being a young and/or male dog and social behaviours linked with poor health conditions and poor living environments of dog owners. Ecological factors influencing *E. multilocularis* transmission encompassed population densities of foxes and rodents, predator-prey relationships, geographical characteristics, climate conditions and the movement of foxes towards urban areas. These findings are important, as intervention to control echinococcosis requires intervention in animal populations.

Echinococcosis infection constitutes a significant financial constraint derived from human health costs and livestock production losses. The global burden of CE and AE has been calculated to be of approximately 1 million and 600,000 DALYs respectively [16,17]. In addition the economic burden of CE on the global livestock industry has been estimated at over $2 billion per annum [16]. Despite the substantial socioeconomic impact, CE and AE remain neglected zoonoses [18].

A sound understanding of the epidemiology of infection in animals is a key factor in limiting the transmission to humans. Controlling the parasitic infection in animals is crucial to reduce the incidence of human disease. The study of *Echinococcus* transmission on animal hosts draws heavily on statistical and epidemiological models. Modelling enhances our epidemiological understanding of parasite transmission allowing predictions to be made and thus, the evaluation of potential control strategies in a cost-effective way. Moreover, the World Health Organization has recently included human echinococcosis within the group of neglected tropical diseases, and recommends a veterinary public health strategy as part of an effective control approach [19]. However, to the authors' knowledge, a study summarizing risk factors that have been found to have significant association with *Echinococcus* infection in animals is lacking. The purpose of this review is to provide an exhaustive summary of determinants that were found to be significantly associated with *Echinococcus* infection in animal hosts, in order to better understand the parasite epidemiology. This knowledge will assist in the design of effective control programmes to reduce transmission to humans.

Materials and Methods

The objective of this study is to review the current state of understanding on risk factors for echinococcosis in animals by presenting significant results from epidemiological associative studies collected in a systematic way. Associative studies determine the strength of association between disease occurrence and suggested risk factors. These studies employ a number of commonly used statistical techniques defined in Table S1 (Table S1).

Principal data sources selected to carry out the literature search included six bibliographic databases: PubMed, Scopus, Web of Knowledge, Cab Direct, Science Direct and Google Scholar. The computer search was not constrained by language or date, although the eligibility criteria were restricted to 5 languages. The online search was conducted by combining topic-related keywords using Boolean operators. The asterisk (*), when used, expanded the search by looking for words with similar prefixes (i.e. echinococc* will search for echinococcus, echinococci, echinococcosis, echinococcoses). Different combinations were tailored for each electronic database in order to narrow the amount of results retrieved but at the same time maximizing the number of relevant studies. The last online search was performed on the 15th October 2012. Table 1 illustrates the number of papers identified in each database.

At the first selection stage, the titles and/or abstracts of the studies retrieved were screened for relevance to the topic. At the second stage, the full texts of retained documents were examined to detect eligible studies. The eligibility criteria encompassed available publications in certain languages (English, Spanish, Italian, French and German), type of study (peer-reviewed epidemiological analytical studies), methodology applied (associative statistical methods) and results (statistically significant findings). Remaining records were combined to eliminate duplicate publications. Furthermore, the reference lists of the selected studies were examined as a method to supplement the electronic searching.

Data were extracted from the selected studies by filling tables containing the four following sections: article reference, study information, statistical method applied and significant factor/s reported. Data on study characteristics included: study description, geographic location, type of animal host studied, sample size and statistical analyses performed. If the analysis was undertaken with multiple explanatory variables, only variables that remained significant were included. Disease determinants were reported along with their significant p-values ($p<0.05$) or equivalent measure of goodness of fit, such as the Akaike information criterion (AIC), the coefficient of determination (R^2) or 95% confidence intervals, retrieved from tables and text of primary articles. Furthermore, measures of association between significant risk factors and infection are also reported when available (e.g. Odds ratio).

The systematic review followed PRISMA guidelines and a PRISMA check list is provided as supplementary material (Checklist S1).

Results

The literature search yielded 1,935 potentially relevant references (see Table 1). After the first screening by title and/or abstract, 568 remaining publications were assessed by a full text examination. Of the 369 articles discarded during this second selection, the two most common reasons for exclusion were if only measures of disease occurrence (prevalence) were reported and if there were a lack of statistically significant factors. Other reasons for exclusion included language, presenting non-original results, article availability or when the statistical method used for the analyses was not associative. A total of 100 references were presented in the review tables, including 23 additional articles retrieved from the screening of references lists of the eligible papers. The flow diagram in Figure 1 shows the review process.

Table 1. Search strategies and results for 6 electronic databases[1].

Database	Search strategy	Results
PubMed	"echinococcus"[Mesh Terms] AND "epidemiologic factors"[MeSH Terms]) AND "animals"[MeSH Terms]	130
Scopus	TITLE-ABS-KEY (echinococcus AND epidemiolog* OR factor* AND dog* OR fox* OR livestock) AND SUBJAREA (mult OR medi OR vete OR heal)	466
Web of Knowledge	Topic = (echinococcus) AND Topic = (epidemiolog* factor*) AND Topic = (animal*)	302
Cab Direct	(echinococc*) AND (epidemiolog*) OR (factor*) AND (dog*) OR (fox*) OR (animal*)	366
Science Direct	(echinococc*) AND (epidemiolog* factor*) AND (animal*) AND LIMIT TO (topics, "echinococcus granulosus, echinococcus multilocularis, veterinary parasitology, cystic echinococcosis, hydatid disease, tropical medicine, alveolar echinococcosis, hydatid cyst, Infectious disease, parasitic zoonosis, red fox")	301
Google Scholar (1)	TITLE-(Echinococcus multilocularis foxes)	130
Google Scholar (2)	TITLE-(Echinococcus granulosus dogs)	240

[1]Last search performed on the 15th October 2012.
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This review presents some limitations with regards to missing publications, language bias and publication bias. The combination of terms entered in each individual computer search aimed to retrieve as many relevant publications as possible but at the same time tried to narrow the amount of results. Hence, it is highly possible that relevant papers, which did not contain in their titles or abstracts the key words used in our search, may have been overlooked. In addition, just around 5% of the articles selected were not written in English, indicating a major bias towards English publications. Furthermore, about 95% of selected papers were obtained through electronic search. Thus, a bias towards articles published online has to be acknowledged. Additionally, this review has a strong bias towards articles reporting positive findings. Nevertheless, it was decided from the beginning that significant findings were a requirement for eligibility of inclusion. Finally, it is worth remembering that, in research, significant results are the ones reporting p-values less than 0.05. Yet, this is just an agreed threshold to have a convenient and standardised way to assess the statistical significance of an effect.

In addition, the majority of the studies included in this review were cross-sectional studies reporting *Echinococcus* infection and associated risk factors at a specific point in time. These types of studies can be subjected to selection and information bias. Common sources of potential bias affecting *E. granulosus* studies can be borne from recall errors or non-responded questionnaires from dog owners, non-randomly selected animals (e.g. abattoir studies) or misclassification bias due to imperfect sensitivity and specificity of the diagnostic test used (e.g. aerocoline purgation or coproantigen ELISA). Common sources of potential bias in *E. multilocularis* studies included the selection of sampled animals being based just on availability (e.g. foxes shot or found dead) and misclassification when the diagnostic test used was other than necropsy. Although acknowledging potential bias, no studies were excluded for qualitative reasons.

Associative models for *E. granulosus* in definitive hosts

Dogs. The predominant life cycle of *E. granulosus* takes place in a synanthropic cycle with domestic dogs (*Canis lupus familiaris*) as definitive hosts and livestock animals as intermediate hosts. A number of factors have been found to influence the frequency and intensity of canine echinococcosis. The most important of these is the potential access that dogs have to uncooked and infected offal. The determinants that might increase access to offal include food sources, access to the location where animals are slaughtered, access to livestock rearing areas and carcasses, non-urban location of dogs, whether dogs are free to roam, the type of dog, the knowledge of the owners about echinococcosis and their socio-economic background. Other determinants of canine echinococcosis include the age and gender of the dogs, and if the dogs receive anthelmintic treatment.

The feeding of domestic dogs with infected offal perpetuates *Echinococcus* transmission (Table S2). Dogs known to eat raw offal or infected viscera were reported more likely to be coproantigen positive for *E. granulosus*. [20,21]. Similarly, activities that prevent the consumption of livestock offal by dogs, such as the proper disposal of animal carcasses by incineration/burial or not performing home slaughtering, were found protective factors for dogs' infection [21,22].

Likewise, dogs with more possibilities to have contact with livestock were more likely to become infected. Dogs from a semi-nomadic pastoral community in north-west China presented more than 2.5 times higher coproantigen positivity in the winter area than in summer pastures [23], possibly due to greater availability of offal when animals are slaughtered. Farm dogs and sheepdogs showed higher infection rates than other type of dogs [20,24,25]. In Patagonia, Argentina, a positive correlation between livestock premises showing higher canine coproantigen positivity and their number of reared sheep was found [26]. Similarly, dogs living in rural communities, or with access to fields, presented a higher risk of infection compared with urban dogs [22,24,25,27,28]. Nevertheless, a study reported lower odds of a dog being copropositive in rural sites and towns compared to cities, although the same study found higher prevalence in dogs from urban households located in the periphery of a city, near to rural areas [22]. In Tunisia dogs located within 1 km of a refuse dump presented high infection rates [29].

The ability of dogs to roam freely was one of the most commonly reported risk factors for *E. granulosus* infection. Several studies reported that dogs which were free to roam presented an increased risk of being coproantigen positive, compared to indoor or chained dogs that were restrained most of the time [21,27,30–33]. Likewise, stray dogs showed greater intensity of infection compared with domesticated dogs [34].

Several studies reported a higher risk of *E. granulosus* infection in young dogs compared to adults (Table S3). Higher canine prevalence was commonly reported in young animals (<2 years)

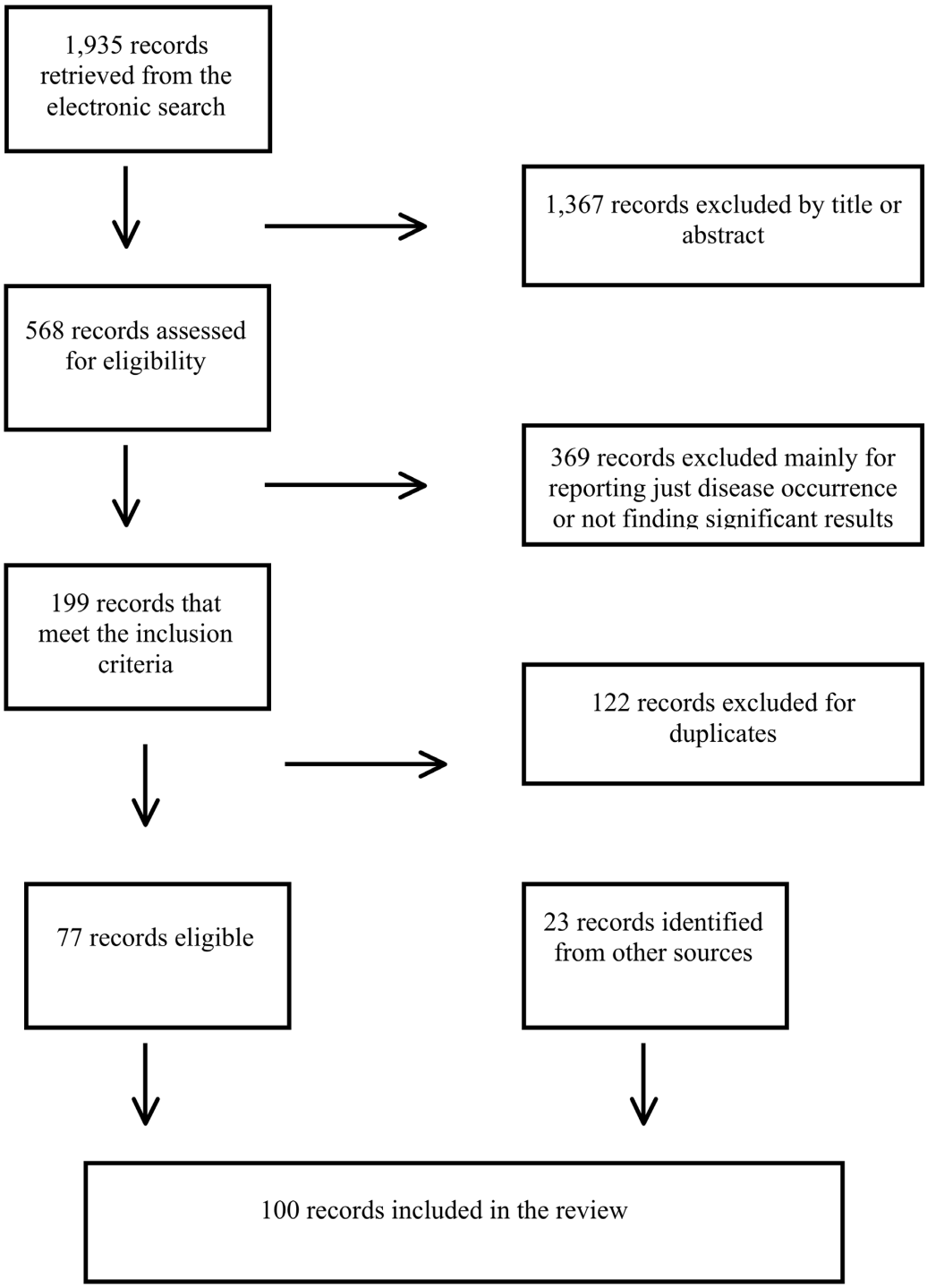

Figure 1. Literature search flow diagram.
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[22,35]. Likewise, dogs older than 5 years showed lower coproantigen positivity, and even lower parasite burden, compared to younger groups [21,24,34].

Although numerous studies recorded higher prevalences in males compared to females, just one study was found to report this difference as significant [27].

Seven retrieved studies supported the existence of an increasing risk for canine infection and some socio-economic factors associated with dog ownership (Table S4). Risk factors for *E. granulosus* infection were associated with the dog owner's lack of knowledge about parasite transmission or deficiencies in the anthelmintic treatment [22,24,27,31,33]. Additionally, the cultural and economic background of the owners was found to be related to infection risk in dogs. In Cyprus, the percentage of Turkish Cypriots in the village explained, approximately 9% of the total variance in the prevalence of canine echinococcosis [36]. Likewise, the Maori population represented a major obstacle for the success of an echinococcosis campaign in dogs in New Zealand [37].

Associative models for *E. granulosus* in intermediate hosts

Livestock. The transmission cycle of *E. granulosus* relies primarily on the domestic cycle where farm species act as intermediate hosts. Principal determinants of livestock infection found in the literature encompassed the level of environmental contamination with parasite eggs and age of the host, among others (Table S5).

Significant differences in prevalence of cystic echinococcosis between study locations or different livestock origin have been repeatedly reported [38–45]. Seasonal variations in hydatidosis prevalence were also recorded through abattoir meat inspection [46,47]. Other environmental factors found associated with CE in livestock were high altitudes and increasing annual rainfall [44,48].

The age of the host has been largely recognised as an infection determinant for many farm species. Numerous studies have recorded higher hydatidosis prevalence in old animals compared to young ones [41,43,49–56]. Small ruminants (sheep and goats) equal or older than 3 years old were also found to be 1.6 times more at risk compared to the younger groups [57]. Additionally, an increase of cyst abundance has been reported in older age groups of farm animals [47,55,58,59].

The gender of the intermediate host has also been identified as a possible determinant of CE, although reports were inconsistent. In a large slaughterhouse survey in Saudi Arabia, females were found significantly more likely to be infected than males for cattle (OR 1.76; 95%CI 1.27, 2.43) and sheep (OR 1.21; CI 1.01, 1.44) [47]. Females were also reported showing higher prevalence than males in eastern Libya [54], Kuwait [60], Iran [61] and in China [62]. Contrarily, a study carried out in Ethiopia revealed that small male ruminants were significantly more susceptible to infection compared to the females [51].

Significant differences in CE prevalence were consistently found among host species. However, reported studies differ on which farm species presented the highest rates. Small ruminants have frequently been observed showing high rates of infection [47,63], with sheep registering higher risk of infection compared to goats [51,54,57]. Cattle have also been identified in many studies as bearing the highest prevalence of CE of those observed in farm species [40,44,48,64–66]. A study reported camels as the domestic intermediate host most likely to be infected, although cattle were recorded with the highest cyst intensity [47].

Finally, farm location and management factors were reported to be associated with hydatid disease in livestock. Local cattle breeds showed higher cyst prevalence than crossbreeds in an Ethiopian study [67]. Pigs reared in intensive conditions reported significantly lower prevalence compared to pigs reared in free-range conditions or on family farms [50,68]. While sheep and goats from mixed farming systems showed higher rates of hydatid infection compared to small ruminants from pastoral systems [51]. In a geo-referenced study carried out on cattle and water buffalo farms, showed that the distance from positive testing cattle farms to sheep farms were significantly lower than for positive testing water buffalo farms. Cattle had higher prevalences (20.0%, 95%CI 18.5–21.6%) than water buffaloes (12.4%, 95%CI 10.0–15.4%) [64].

Wild intermediate hosts. CE has been recorded in a large number of wild animals, even although wildlife studies rarely report more than point prevalence estimates. A publication was found to report that kangaroo females were twice as likely to be infected as males [69]. Other studies reported that there was an increasing prevalence and intensity of cysts in correlation with an increase in the density, and age, of the moose population [70,71] (Table S6).

Associative models for *E. multilocularis* in definitive hosts

Foxes. In contrast with the domestic cycle of *E. granulosus*, the transmission of *E. multilocularis* is primarily supported by foxes and small mammals [72]. Although the Red fox (*Vulpes vulpes*) has been identified to be the most common definitive host, other fox species such as the Arctic fox (*Vulpes lagopus*, formerly *Alopex lagopus*), the Corsac fox (*Vulpes corsac*) or the Tibetan fox (*Vulpes ferrilata*), are also susceptible to infection [73].

Factors identified in this review as contributing to the infection rates of *E. multilocularis* in foxes include; host population dynamics, interactions with prey animals, spatial distribution, seasonal changes and age. As such factors are interrelated it can be challenging to resolve independent risk factors for infection.

There is extensive literature linking young foxes with *E. multilocularis* infection (Table S7). Many epidemiological studies have reported a higher prevalence and/or abundance in juvenile foxes (<1 year old) compared with adults [74–80]. However, some researchers have found that this relation between parasite infection and host age is influenced by other factors. In Germany, under high-endemic conditions young foxes were found to be more frequently infected than adults whereas in low-endemic areas infection rates were higher in adults (OR 2.25, 95%CI 1.26–4.02) [81]. In Switzerland, seasonal changes of prevalence were found to be more pronounced in juveniles than in adults (i.e. summer/autumn×juvenile vs. winter×adult (OR 0.36, 95%CI 0.14–0.91). Whereas prevalence differences that related to the type of urbanization level were more pronounced in adults (i.e. urban×juvenile vs. peri-urban×adult (OR 4.76, 95%CI 1.26–17.39) [82].

There is less scientific evidence to support that being a male or female fox act as an independent variable influencing the infection status of the animal. Just one study identified being a male as a significant regressor parameter associated with the mean parasite abundance in foxes [83].

Environmental factors seemed to play a critical role in *E. multilocularis* infection in foxes (Table S8), resulting in a heterogeneous geographical distribution of the parasite [81,84–86].

Specific geographic-related features can act directly upon parasite transmission. For example, in Germany significant differences in prevalence were reported between 3 different locations (i.e. Zone1 vs. Zone2, OR 2.64, 95%CI 1.92–3.64 or Zone1 vs. Zone3, OR 4.9, 95%CI 3.12–7.73) [81]. In the same country, the highest parasite burdens were found in foxes from regions with a high quota of agricultural land and precipitation [87]. In France, mid-altitude areas with a high proportion of

permanent grassland showed higher fox prevalence when compared with low altitude sampling locations [88]. Likewise, regional meteorological conditions, such as low temperatures or high annual precipitation, have been reported as being associated with the infection rates in foxes. For instance, a significant correlation was established in Slovakia between *E. multilocularis* prevalence/abundance and the increasing mean annual rainfall [89,90]. Inversely, a negative association between the infection of foxes and annual temperature was recorded in the German Saxony [91].

Similarly, infection rates in foxes have been documented to vary between seasons [92,93]. In Belgium, foxes collected in summer and autumn were more often infected than the ones collected in winter and in spring [93]. Sometimes these seasonal variations in prevalence were found to also relate to other factors. In Zurich, Switzerland, seasonal changes of prevalence were observed to be more pronounced in juveniles (<1 year old) than in adult foxes (i.e. Summer/autumn×juvenile vs. winter×adult, OR 0.36, 95%CI 0.14–0.91) [82]. Again in Zurich, significant seasonal differences could only be established in sub-adult male foxes caught within the urban area [76]. Variations in prevalence between seasons and geographic location were also found to be dependent on host age in western Switzerland [75].

As previously mentioned, the spatial distribution of *E. multilocularis* in foxes was found to be linked to regional geographic and climatic conditions (Table S9). Several spatial studies have identified disease clusters or locations where foxes presented higher parasite prevalence [91,94–96]. Spatial studies on *E. multilocularis* in foxes have also helped to establish associations between location of infection, landscape characteristics and ecological factors. In France, the percentage of grassland was associated with fox coproantigen distribution [97]. In Germany infected foxes were more frequently caught near humid areas and pastures [98]. Whereas, in Svalbard (Norway), positive infected faeces from the artic fox were confined within the habitat of the only intermediate host available, the sibling vole (*Microtus levis*) [99].

Transmission dynamics of *E. multilocularis* depend directly on the densities and predator-prey relationship between definitive and intermediate hosts. These two factors differ greatly among the level of urbanization in different areas (Table S10). Despite a higher prevalence in foxes from rural areas when compared with urban areas [100], there is a high infection pressure frequently reported in the periphery of the cities [78,101]. Some studies found that the association between infection status and type of urbanization zone was related to other variables such season or age of the host. In Zurich, higher infection rates during winter were recorded in rural and peri-urban foxes compared with urban animals [76,102]. In the same city, prevalence variations between urban types were more pronounced in adults than juveniles (i.e. Spring×juvenile vs. peri-urban×adult, OR 0.23, 95%CI 0.06–0.89) [82].

Many authors have highlighted the importance of the availability and predation level on potential intermediate hosts for the successful transmission of *E. multilocularis*. The relationship between parasite prevalence in foxes and vole abundance was reported in Hokkaido (Japan), where infection rates in foxes were proved to be dependent upon the current-year abundance of voles [103]. Likewise, several publications have evidenced a significant correlation between parasite prevalence in foxes and the density [89], prevalence [93] and predation of potential intermediate host populations [104]. Additionally, the infection level in foxes is also dependant on fox population density [105].

Other carnivores. Some wild carnivores, members of the family Canidae and Felidae, can harbour *E. multilocularis*. Disease determinants for *E. multilocularis* infection in definitive hosts, other than foxes, appeared to be associated with greater exposure to infected intermediate hosts (Table S11). As in foxes, canine infection was linked with the abundance and availability of potential intermediate hosts [106,107]. Dogs that preyed on rodents were more likely to be infected [108]. Similarly, non-restrained dogs or hunting dogs were identified as having greater exposure to rodents, and thus, to infection [12,109]. In Germany, regional differences in canine prevalence were observed between the north and the south [110]. Other carnivores, such as racoon dogs, showed seasonal variations in prevalence [83] whereas higher prevalence was recorded in young (<1 year old) [111] and male coyotes [112].

Associative models for *E. multilocularis* in intermediate hosts

Voles. More than 40 species of small mammals (rodents and lagomorphs) can act as intermediate hosts for *E. multilocularis* [10]. Among them, grassland rodents (i.e. *Arvicola terrestris* or *Microtus sp.*) have been identified as playing an important contribution to the diet of foxes and on cestodes transmission [113].

The risk of *E. multilocularis* infection in rodents is influenced by ecological and environmental factors that ultimately shape their numbers and age-structure (Table S12). Voles' annual population fluctuations had a significant effect on the yearly prevalence recorded in *A. terrestris* [114]. Environmental factors such as type of habitat or climatic season and their derived interaction terms, were found to explain much of the variance observed in parasite prevalence in the deer mouse (*Peromyscus maniculatus*) [115]. Low average day temperatures significantly increased the infection risk in *A. terrestris* [116]. Geographic location and sampling site have also been reported to be associated with infection rates in voles [102,116–118]. Prevalence of *E. multilocularis* in rodents has been frequently associated with their increasing length and body size, which is linked to maturity and age [117–119]. Adult voles have frequently shown higher prevalence compared to sub-adults or juveniles [93,102,116].

Table 2 presents the summary of key findings reported in this review.

Discussion

Human echinococcosis is a widely distributed parasitic infection, which despite adding a significant health and economic burden to the human race, is still a neglected disease [120]. A sound understanding of the epidemiology of *Echinococcus* in animal hosts is essential for designing an effective control programme [18]. To the authors' knowledge, this is the first study to systematically collect data on the infection determinants of *Echinococcus* in animals.

CE is a widespread chronic zoonosis, and domestic dogs have long been identified as the main infection source for humans. Dogs acquire *E. granulosus* through the ingestion of viscera from infected intermediate hosts. Factors facilitating the contact of dogs with raw offal are potential determinants for canine infection. Dogs from a semi-nomadic pastoral community showed higher infection levels in winter when higher numbers of livestock are slaughtered for the winter provisions [23]. Being a farming dog has been established as a risk factor for *E. granulosus* infection since they usually have higher contact with livestock, which can be seen as a proxy for scavenging on infected carcasses [20,24,25]. Hence, the risk of *E. granulosus* infection in dogs is commonly higher in rural areas [28]. However, high infection rates have also been recorded in dogs from the borders of urban areas. The continuation of the practice

Table 2. Key findings.

Causative agent	Host	Risk Factors
E. granulosus	Dog (definitive host)	- Feeding with raw viscera, being a farm, rural or stray dog or being untied or free to roam
		- Being a young and/or male dog
		- Dog owner's lack of knowledge about hydatid disease and the lack of deworming treatment in dogs plus the owners' ethnic origin (linked with poor health education and deprived living conditions)
E. granulosus	Domestic livestock (intermediate hosts)	- Increasing hosts' age, geographical location, meteorological conditions, female gender, host species and type of farming management
E. granulosus	Wild life (intermediate hosts)	- Hosts' age, female gender and hosts' densities
E. multilocularis	Fox (definitive host)	- Being a young and/or male fox
		- Climatic conditions and geographic location (marked spatial distribution)
		- Host population dynamics and interactions with intermediate hosts (rodents), frequently influenced by urbanization level
E. multilocularis	Other canids (definitive host)	- Feeding with raw viscera, being hunting dogs or free to roam and availability of rodents
E. multilocularis	Rodents (intermediate hosts)	- Increasing adult age
		- Meteorological and geographical conditions
		- Rodent's densities

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of home slaughtering in the periphery of some cities might explain these findings [22]. Similarly, dogs allowed to roam [27,30–32] or stray dogs [29,34] have also been identified as presenting higher infection risk as they have increased possibilities of finding and ingesting raw carcass meat and offal of fallen livestock. In contrast, dogs that cannot roam freely, like guard-dogs or household pets, commonly present lower infection rates, which may be due to a diet comprising mainly of cooked food or kitchen scraps [24] that are unlikely to contain viable hydatid cysts. However, such differences in relative infection rates may also be explained by the fact that dogs which are allowed to roam free are less likely to receive regular anthelmintic treatment than, for example, dogs kept as pets or guard dogs [32].

Multiple studies have found that *E. granulosus* prevalence and/or abundance is higher in young dogs compared to adults [21,22,24,34], supporting the hypothesis that protective immune responses increase with the age of the host [121]. However, changes in infection pressure due to behavioural differences related to dog's age cannot be ruled out [122]. In addition, prevalence studies have observed higher numbers of infected male dogs compared to females [22,27]. A plausible reason might be that male dogs tend to break away from the pack and explore larger areas than females, due to their tendency towards territorial behavior and to go hunting [12].

Human behavior has also been recognized as playing a key role in the perpetuation of echinococcosis transmission [123]. This behaviour is closely related to human cultural and economic backgrounds [124]. The use of epidemiological techniques and anthropologic knowledge has served in the past to highlight the reasons for the distribution of echinococcosis [125]. Studies in Table S4 that reported dog owners' ethnicity as being related with canine infection rates also found a higher number of dogs per owner, lower levels of education and lower standards of animal care, when compared with other ethnic groups [36,37]. Thus, this variable may act as a confounder for other risk practices. Likewise, the changes in agricultural practices following the collapse of the Soviet Union may partly explain the increase in echinococcosis in Central Asia [3]. The social and economic changes brought after the collapse of socialist administration, such as the return to small private farms, the proliferation of the clandestine slaughter or the lack of anthelmintic dog treatment, are associated with a substantial increase in echinococcosis [25].

There are numerous studies reporting high parasite prevalences in wild canids [126,127], although none of these reported statistically significant associations with potential disease determinants. For instance, *E. granulosus* was a frequent helminth parasite found in wolves (*Canis lupus*) presenting a meta-prevalence above 19%, although the tapeworm was more commonly reported in the Nearctic wolf populations compared to the Palaearctic [128]. The predator-prey relationship between wolves and moose (*Alces alces*) in North America has been documented for a long time [129]. More recently, Joly and Messier suggested that *E. granulosus* might have an influence in the regulation of the intermediate host populations by increasing the risk of predation of heavily infected moose by wolves [130]. In North America, *E. granulosus* has not only been reported in wolves but also in coyotes (*Canis latrans*) [127]. In Kazakhstan, a prevalence of 19.5% (95%CI 8.8–34.9) has recently been reported in wolves [131]. In Australia, *E. granulosus* is widespread in wild dogs (dingoes (*Canis lupus f. dingo*) and dingo/domestic dog hybrids) and is occasionally seen in foxes (*Vulpes vulpes*) [126]. In Africa infections have been found in golden jackals (*Canis aureus*), silver backed jackals (*Canis mesomelas*) and African wild dogs (*Lycaon pictus*) [126]. Additionally, there is experimental evidence of successful transmission between wild and domestic hosts [132]. Thus, wild hosts represent an important reservoir for *E. granulosus* transmission particularly where there is an overlap between human and wild animal habitats [133].

A wide range of domestic ungulates such as sheep, goats, cattle, pigs, equines and camelids serve as intermediate hosts for the larval stage (metacestode) of *E. granulosus*. The majority of risk factor studies in livestock species reported cross-sectional data from abattoir surveys. Environmental temperature and humidity are major influencing factors for livestock infection [134]. Low temperatures and high rainfall permit longer viability of eggs in the environment, a critical factor when ensuring the perpetuation of the parasite cycle. Hence, several studies have reported higher

levels of CE in domestic livestock in areas presenting these environmental conditions when compared with warmer and drier sites [38,47,135]. The age-dependent increment in infection rates has been reported in many studies supporting the apparent lack of parasite-induced immunity in naturally infected intermediate hosts [134]. Therefore, both prevalence and abundance of hydatid cysts increase with age in intermediate hosts [134]. Alternatively, particular husbandry practices associated with age could explain the large prevalence reported in some farm species, like cattle and camels in Ethiopia [48].

Experimental studies have suggested that parasite survival may be longer in females due to the potential link between sexual hormones and the response of the immune system [136]. In Ethiopia male small ruminants were reported with higher infection risk compared to female [51], although this study may be biased as larger numbers of males than females were included in the sampled population. An alternative explanation may lie in the fact that females are slaughtered at older age as they are retained for reproductive purposes [47,54]. Therefore, a longer life expectancy increases the probability of exposure and infection. Consequently, higher prevalences are usually found in older animals [54,137].

Sheep frequently present the highest infection rate [54,138] and are often the most important intermediate hosts for *E. granulosus* [2]. However, cattle and camels are normally sent to the abattoir at an older age than other ruminants, and hence have an increased risk of exposure to *E. granulosus'* eggs during their lifetime. Goats show lower infection rates, possibly because they are browsers and eat the most distal parts of plants where there are fewer eggs. Moreover, these eggs commonly have a greater exposure to hostile environmental conditions, and thus show a reduced infective capacity [139]. The difference in prevalence between host species could also be a result of the existence of different strains of *E. granulosus* morphologically and biochemically adapted to each farm species [48]. Human activities play also a critical role in the persistence of *E. granulosus* in farm species. Different management practices might be behind the infection differences showed between family and industrial pig farms [50,68]. Similarly, the local cattle breeds in Ethiopia presented higher infection rates than the crossbreeds presumably because crossbreeds are frequently kept indoors whereas local breeds are pasture-grazing animals [67]. In Sardinia, the highest sheep prevalences were associated with farms whose owners admitted throwing the viscera into the trash/garbage and feeding their dogs with offal [140].

Wild animals can also act as intermediate host for *E. granulosus*. In North America, hydatid cysts have been found in elk (*Cervus canadensis*), moose (*Alces alces*), red deer (*Cervus elaphus*), caribou (*Rangifer tarandus*) and various species of deer [127]. In Canada, researchers have reported an age-related hydatid prevalence and intensity; suggesting the absence of immunity in wild intermediate hosts [70,71]. In the same region, *E. granulosus* infection in moose was also related with increasing population density. Authors suggested that higher numbers of moose were linked with a more intense wolf predation pressure, and hence these moose were exposed to a higher environmental parasitic contamination [70]. In Africa, herbivores such as warthogs (*Phacochoerus sp.*), hippopotamus (*Hippopotamus amphibius*), giraffes (*Giraffa camelopardalis*), zebras (*Equus quagga*, *Equus zebra*) or impalas (*Aepyceros melampus*) are known to be susceptible to CE [141]. In Australia, CE has been reported in native mammals belonging to the Macropodidae family, such as kangaroos (*Macropus giganteus*, *Macropus fuliginosus*) and wallabies (*Wallabia bicolor*, *Macropus rufogriseus*), along with other marsupials such as wombats (*Vombatus ursinus)* [133]. The higher hydatid infection and intensity showed in eastern grey female kangaroos compared to males were suggested to be age-related and attributed to a higher human hunting pressure on larger animals, older males preferentially. Thus, female kangaroos live longer and hence are more likely to present higher infection and intensity rates than males [69].

E. multilocularis is endemic in foxes in large areas over the northern hemisphere [17]. In humans the larval stage of *E. multilocularis* causes AE, a space-occupying lesion, which is lethal if untreated. Association between parasite infection/burdens and young age in foxes have been frequently reported [74–76]. Nevertheless, differences in prevalence between juveniles and adults have not always been statistically significant [77,101]. Investigators have not arrived to a conclusive biological reason for finding juveniles more frequently and/or intensively infected than adults. A proposed explanation is that adult foxes might acquire partial immunity after repeated exposure [75,76] and young foxes could be more susceptible to infection when they assume a similar diet to that of the adults [81]. Endemic levels might also contribute to the differences in prevalence reported by host age [81] as low infection pressure can lead to an upward shift of the age at which protective immunity is acquired. This is known as "the peak shift" [142]. Only one study was found reporting a significant association between fox gender and parasite abundance. Nevertheless, male foxes tend to expand their territories further than females, and thus, they can play a significant role in dispersing the parasite when they are heavily infected [76].

The spatial distribution of *E. multilocularis* infection in foxes comes as a result of a combination of multiple ecological factors. Landscape features and regional climatic conditions not only affect the viability of *E. multilocularis* eggs in the environment but also shape the type of biodiversity given in a region, such as intermediate host populations, which determines parasite transmission. In France, the percentage of grassland was associated with fox coproantigen distribution, possibly related with sudden large increases in rodent populations known to occur in these areas [97]. Additionally, intensive land-use may lead to lower levels of water in the soil hampering the survival of parasitic eggs in the environment [81] whilst regions with high levels of soil humidity (e.g. pastures) present favourable conditions for the survival of the oncospheres outside the host [98].

Regional meteorological conditions contribute significantly to the spatial patterns of infection in foxes. *E. multilocularis* eggs are highly sensitive to both desiccation and high temperatures [143]. Consequently, infected foxes are more frequently found in areas with humid conditions [98]. Similarly, seasonal variations in temperature and precipitation influence the availability of definitive and intermediate hosts and the survival of the parasitic eggs in the environment. This seasonal prevalence fluctuation has been found related with factors such as the host's age [75,82].

Transmission dynamics of *E. multilocularis* depend directly on the predator-prey relationship of their two hosts [10], which in turn respond to environmental conditions among other ecological factors. Local geographic and climatic conditions affect fox and rodent densities, resulting in marked spatial differences in parasite distribution among regions and seasons [75]. In Germany infected foxes were more frequently caught near humid areas and pastures that not only permit survival of oncospheres but also offer a suitable habitat for muskrats (*Ondatra zibethicus*), a susceptible intermediate host [98].

Furthermore, changes in fox population demographics can come as a result from human-related activities, like the progressive expansion of urban areas. In the UK, the increase of fox densities in some cities is believed to be a consequence of the construction of large residential suburbs highly suitable for foxes [144]. The same trend has also been reported in several European cities following

the fox population growth after the successful vaccination campaign against rabies [76,145]. Some other suggested factors responsible for this phenomenon are the greater availability of food (anthropogenic food), the availability of shelter and the lower hunting pressure found in human settlements [145,146]. Moreover, high infection rates of *E. multilocularis* have been recorded in foxes close to urban settlements [76,102]. The increase of fox densities together with the high parasite rates found in foxes near to the edges of cities might have resulted in higher environmental contamination [146]. However, this potential risk of infection may not be of importance as low prevalences in foxes have been reported in city centres compared to peri-urban or rural foxes [78,101]. The scarcity of suitable intermediate prey-hosts in the urban centers and the increased availability of anthropogenic food might have contributed to this low infection rate [82,101].

In addition to foxes, other members of the family Canidae, such as domestic dogs (*Canis lupus f. familiaris*), wolves (*Canis lupus*), coyotes (*Canis latrans*) or raccoon-dogs (*Nyctereutes procyonoides*), are also susceptible to be infected by *E. multilocularis* [147]. Likewise, some members of the family Felidae, such as wildcats (*Felis silvestris*) or domestic cats (*Felis silvestris f. catus*), can harbour *E. multilocularis* worms, although, cats appear to be a poor host for *E. multilocularis* [147]. In contrast, domestic dogs are an important definitive host and may contribute to the maintenance of *E. multilocularis* in a synanthropic cycle, particularly in certain rural communities [148]. The presence of *E. multilocularis* in dogs has been previously reported in endemic areas [12,149]. Some of the risk factors associated with the acquisition of *E. multilocularis* are similar to those found for *E. granulosus*, such as non-restrained dogs or being a dog fed with uncooked viscera [12,108]. As with *E. granulosus*, untied dogs have more possibilities of hunting small mammals and, thus have greater exposure to infection [12,109]. Positive coproantigen results were mainly reported in working dogs such as hunting, guard or shepherd dogs [108] that presumably are more likely to roam freely and less likely to be dewormed regularly. The high numbers of positive dogs found in southern Germany might be related with high parasite prevalences presented in fox populations in the same region [110]. The role of domestic dogs in the transmission of *E. multilocularis* to humans appears to be of importance in certain communities where dog ownership, number of dogs owned or contact with them were found associated with human AE risk [14,150].

The predator-prey dynamics between definitive and intermediate hosts are a key determinant driving *E. multiocularis* transmission [113]. This relationship depends on the host population densities and structures, which are directly influenced by ecological interacting factors such as availability of food, dispersion, reproduction and survival trends [151]. Rodent species are often found in specific landscapes, such as grassland areas, where food and cover are abundant. A hypothesis suggests that the ratio of these optimal habitats can influence the probability of arvicolids undergoing multi-annual cycles [152]. High prevalences of *E. multilocularis* have been reported in foxes in areas presenting a high ratio of grassland [113]. Hence, landscape characteristics contribute to population dynamics of arvicolid species and predator–prey interactions, and ultimately may influence parasite transmission [153]. The risk of *E. multilocularis* infection in rodents is also reliant on local meteorological conditions [143]. Additionally, vole populations commonly present a seasonal reproduction pattern starting in early spring and continuing until later into the autumn. Similarly, their age-structure is also closely dependent to seasonal oscillations, showing a higher proportion of adult voles in spring due to the decreased reproduction during winter [116]. Several studies reported an increasing prevalence of *E. multilocularis* in rodents with age. Therefore, seasonal variations of prevalence in rodents result from shifts in the age structure of voles' populations since a higher number of intermediate hosts are potentially harbouring protoscoleces during winter and beginning of spring [116]. The availability of prey affects the prevalence of *E. multilocularis* in definitive hosts [82,107,118]. Conversely, the number of foxes determines the level of environmental egg contamination in an area, and thus influences the infection rates in small mammals. For instance, in Geneva (Switzerland) low numbers of infected *A. terrestris* were captured in the south-eastern area of the canton where the fox population had decreased due to sarcoptic mange, suggesting that a lower environmental faecal contamination of parasitic eggs might explained the low infection rates recorded in rodents [117].

CE continues to represent a global health hazard affecting approximately over 1 million individuals worldwide [18]. Principal factors reported in this review to be associated with canine infection included potential access of dogs to uncooked livestock viscera, to be an unrestrained young and/or male dog and particular human activities linked with poor health education and living conditions of dog owners. Hence, some recommended measures to interrupt parasite transmission encompass controlled slaughtering of livestock and proper disposal of offal, regular treatment of dogs with praziquantel, vaccination of intermediate hosts and an improvement to the level of health education in poor rural livelihoods [154].

Although AE is confined to the northern hemisphere and generally is a less common disease than CE, is an often-fatal condition when untreated [155]. In addition, the increasing prevalence detected in wild life accompanied by the movement of foxes towards urban areas increases the risk for transmission to humans in Europe [146]. With a complex life cycle involving wildlife hosts, control of *E. multilocularis* remains challenging. Some of the reported ecological factors in this review affecting the transmission dynamics of *E. multilocularis* are hosts' population densities, predator-prey interactions, landscape characteristics, climate conditions and human-related activities. Current control strategies mainly focus on decreasing prevalence on definitive hosts through the distribution of anthelminthic baits for foxes or regular deworming of domestic dogs and preventing infection through education campaigns [154].

The burden of endemic neglected zoonoses falls heavily on rural settings with limited resources [156]. Livestock-rearing communities with subsistence-farming practices are high-risk areas for acquiring CE, while the vast majority of human AE cases are found in certain rural communities in China. Poor health services and shortage of equipment and drugs constrain the diagnosis and treatment of cases, causing premature death or health disabilities. Therefore, it is critical to prevent infection to reduce human incidence. Control of echinococcosis currently relies on the interruption of parasite transmission in animal hosts and, in consequence, a sound understanding of infection risk factors in animals can effectively assist the drawing of a prevention plan. Quantitative frameworks, such as the use of mathematical models, are of great value in the epidemiological research and control of *Echinococcus spp.* in a cost-effective way. This systematic review provides a compilation of epidemiologic factors associated with *Echinococcus* infection in animal hosts identified by the use of associative statistical models in order to assist the design of sound control policies.

Supporting Information

Checklist S1 PRISMA checklist.
(PDF)

Table S1 Glossary of statistical terms.
(PDF)

Table S2 Studies assessing association between *E. granulosus* infection in dogs and potential access to raw offal.
(PDF)

Table S3 Studies identifying significant associations of age/gender and infection of dogs with *E. granulosus*.
(PDF)

Table S4 Studies assessing association between *E. granulosus* infection in dogs and socio-economic factors.
(PDF)

Table S5 Associative studies of *E. granulosus* infection in intermediate hosts.
(PDF)

Table S6 Associative studies of *E. granulosus* infection in wild intermediate hosts.
(PDF)

Table S7 Studies identifying significant determinants of infection of foxes with *E. multilocularis*.
(PDF)

Table S8 Studies assessing association between *E. multilocularis* infection in foxes and environmental factors.
(PDF)

Table S9 Spatial studies of *E. multilocularis* in foxes.
(PDF)

Table S10 Studies assessing association between *E. multilocularis* infection in foxes and host population factors.
(PDF)

Table S11 Associative studies of *E. multilocularis* infection in carnivores, other than foxes.
(PDF)

Table S12 Associative studies on *E. multilocularis* infection in intermediate hosts.
(PDF)

Author Contributions

Conceived and designed the experiments: BOA PRT. Performed the experiments: BOA. Analyzed the data: BOA. Contributed reagents/materials/analysis tools: BOA PRT. Wrote the paper: BOA PRT.

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Paper 2

Dynamics of the force of infection: insights from *Echinococcus multilocularis* infection in foxes

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Dynamics of the Force of Infection: Insights from *Echinococcus multilocularis* Infection in Foxes

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Abstract

Characterizing the force of infection (FOI) is an essential part of planning cost effective control strategies for zoonotic diseases. *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis in humans, a serious disease with a high fatality rate and an increasing global spread. Red foxes are high prevalence hosts of *E. multilocularis*. Through a mathematical modelling approach, using field data collected from in and around the city of Zurich, Switzerland, we find compelling evidence that the FOI is periodic with highly variable amplitude, and, while this amplitude is similar across habitat types, the mean FOI differs markedly between urban and periurban habitats suggesting a considerable risk differential. The FOI, during an annual cycle, ranges from (0.1,0.8) insults (95% CI) in urban habitat in the summer to (9.4, 9.7) (95% CI) in periurban (rural) habitat in winter. Such large temporal and spatial variations in FOI suggest that control strategies are optimal when tailored to local FOI dynamics.

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Introduction

The force of infection (FOI) is a crucial epidemiological parameter and characterizing its dynamics is an essential part of planning cost effective control strategies for infectious diseases [1]. Mechanistically, disease intervention strategies are typically targeted at decreasing the per capita infection rate. If successful, this will then cause a decrease in observed prevalence. As such, quantification of the FOI provides a key measure of efficacy when assessing or comparing interventions [2]. The FOI can be extremely difficult to estimate directly, i.e. observationally, in wildlife populations. Even in human populations this is not without considerable challenges, and requires accurate longitudinal monitoring of the target population in order to capture all new infections which arise [3]. An alternative approach is to estimate the FOI indirectly, through access to prevalence data, in conjunction with either an explicit mathematical model describing the disease transmission processes, or else some assumed disease risk function [4,5].

Foxes are typical definitive hosts for the parasite *Echinococcus multilocularis*, with different rodent species being the primary intermediate host in which the alveolar hydatid cysts grow. In humans, which are aberrant hosts, this parasite causes the important emerging zoonosis alveolar echinococcosis (AE). This is a serious disease with a high fatality rate in the absence of appropriate treatment [6]. In Europe there have been increasing numbers of AE cases reported in the Baltics [7], Poland [8], Austria [9] and in Switzerland [10]: the latter associated with an increase in fox populations. The disease is also emergent in central Asia with a huge increase in the numbers of human cases in Kyrgyzstan recorded in recent years [11]. This disease also has a considerable impact on human health in Western China, particularly on the Tibetan plateau [12]. Alveolar echinococcosis is also an emerging public health concern in North America due, at least in part, to the increasing urbanization of wild canids [13]. Red foxes (*Vulpes vulpes*) are high prevalence hosts of *E. multilocularis* [14], where zoonotic transmission may occur through environmental contamination [15] or through contaminated food [16]. In addition, dogs are susceptible definitive hosts [17] and may be very important for transmission to humans where prevalences in dogs are high, such as in China [18] or central Asia [19]. In Europe, dogs are low pravalence hosts [20], but nevertheless may pose a high risk of introducing the parasite in non endemic countries such as the UK if appropriate treatment is not given when dogs enter the country [21].

In terms of potential control measures for reducing the risk of AE, a number of different studies have investigated anthelmintic baiting in foxes [22]. The impact of such approaches on reducing prevalence appears to strongly depend on the specific design used, in relation to how the baits are delivered and choices of location, and frequency. In Switzerland, year round monthly anthelmintic baiting is an effective control measure in foxes [22]. The *E. multilocularis* transmission cycle is, however, dynamically highly complex with many known temporal-spatial heterogeneities (for example [23]). Adopting, therefore, a baiting strategy in close concordance with FOI dynamics could optimize existing intervention strategies. In planning such intervention studies knowledge of the dynamics and magnitude of the FOI can be invaluable, as this potentially allows the frequency of baiting to be tailored to the changing levels of exposure throughout time and across space. This may enable considerable cost saving, as opposed to, for example, monthly all year round baiting across all habitat types.

Author Summary

Human alveolar echinococcosis (AE) is caused by the fox tapeworm *E. multilocularis* and has a high fatality rate if untreated. The frequency of the tapeworm in foxes can be reduced through the regular distribution of anthelmintic baits and thus decrease the risk of zoonotic transmission. Here, we estimate the force of infection to foxes using a mathematical model and data from necropsied foxes. The results suggest that the frequency of anthelmintic baiting of foxes can be optimised to local variations in transmission that depend upon season and type of fox habitat.

In Switzerland it has been shown that there are considerable differences in the spatial and seasonal distribution of the prevalence of *E. multilocularis* in definitive hosts [14,15] and intermediate hosts [23]. These studies indicated that 129 of 857 *Arvicola terrestris* were infected of which 12 harboured protocolices. Ten of these animals had between 61 and 452,000 protoscolices. Seasonal patterns of infection in intermediate hosts were seen with highest prevalences seen in over-wintered animals. Thus seasonal anthelmintic treatment of foxes, with a focus on the autumn and winter months, is likely to be a more efficient strategy in reducing the parasite biomass [23]. Likewise although fox densities are highest in urban settings, they consume fewer rodents and have a greater reliance on anthropomorphic food supplies compared to rural foxes [24], which is likely to significantly affect transmission dynamics on a spatial scale. Consequently, the intensity of intervention strategies could also be tailored to exploit these spatial differences. Such differences in prevalences clearly indicate that relative differences in the FOI exist between rural and urban areas, and between winter and summer seasons.

We develop a statistically robust quantitative characterization of the FOI for *E.multilocularis* in foxes to address three specific research questions: i) firstly, is the FOI constant or dynamic (with age of the host), and what is its value accounting for complexities such as statistical uncertainty; ii) secondly, how much does FOI vary quantitatively with habitat type, in particular between more or less urbanized regions; iii) and thirdly how much does the FOI of infection vary quantitatively on a temporal basis between winter and summer seasons.

Methods

The key methodological aspect of this study is to identify an epidemiologically useful disease transmission model for *E.multilocularis* in foxes. A model whose structure can be objectively justified, and whose parameter estimates provide tangible insight into the key infection processes. Three sources of information are available to support model development: i) prevalence data from a previously presented observational study [24]; ii) approximate estimates as to likely survival times of *E. multilocularis* in foxes from experimental work [17]; and iii) existing transmission modelling frameworks for *Echinococcus granulosus* transmission in sheep and dogs [25]. Using [25] as a starting point, we identify a process model whose structure is an optimal fit to the prevalence data from [24], whilst making use of the parameter estimates from [17] as expert knowledge. Following [25] we utilize ordinary differential equations (ODEs) to describe the transmission dynamics, and to take advantage of prior knowledge from [17] we adopt a Bayesian paradigm [26] for all model fitting and statistical inference.

Study data

The data to which we fit our transmission models is an extension of that previously described in [14] and [24], and includes only samples taken prior to the anthelmintic baiting intervention described in [27]. Samples were collected from in or around the city of Zurich in Switzerland. Three key variables were utilized: i) presence (absence) of *E.multilocularis* infection based on necropsy (details given in [14,24]); ii) the age of each fox, and following previous studies, and as described in [14], cubs were assumed to be born on 1st April and age determination of foxes sampled after 1st July was done via examination of teeth (details given in [14]). Along with the date of death (which is known as these animals were culled by hunters) and the weight at death, each animal's approximate age in years and days was estimated. The final variable utilized was habitat type, where this comprised three zones reflecting differing degrees of urbanization: urban; border; and periurban. The characteristics of these are described in detail in [27]. The urban zone comprises of mostly residential dwellings with relatively few green spaces, the periurban zone is rural comprising of forests, fields, pastures, and meadows. The border zone separates urban from rural, and was defined as extending 250 meters from the edge of the urban area and into 250 meters of the periurban surroundings. The border zone includes largely residential areas, public spaces, allotments and pastures. The data used in the study is in the Supporting Information Data S1. Out of the $n=458$ foxes aged three years or less in the study data, 160 were sampled in the periurban zone, 167 in the border zone and 131 in the urban zone. The overall observed prevalence across all 458 animals was 42.1%, within the periurban, border and urban zones this was 65.6%, 38.9% and 17.6% respectively. The median age across these 458 animals was 0.80 years. In the periurban, border and urban zones the median respective ages were 0.87, 0.77 and 0.59 years.

Disease transmission model

The most general form of hypothesized transmission model we consider for *E. multilocularis* is given in Figure 1. The structure of this model is based on initial work by [25] which has provided a basis for many subsequent disease modelling studies involving in *E. granulosus* and *E. multilocularis*, (e.g. [5,28]). Figure 1 depicts an intuitively reasonable representation of the possible disease states and flows between them based on current known biology of *E.multilocularis* in foxes. The model dynamics here are over age of the host (foxes), as is typical when modelling *E. multilocularis* or *E.granulosus*. We assume a fully susceptible population at birth, i.e. no vertical transmission and therefore $X_0(a)=1$. This dynamic system can be described in a series of ordinary differential equations (ODEs).

State variables are $X_0(a)$, $X(a)$, $Y_0(a)$ and $Y(a)$, where $X_0(a)$ represents the proportion of hosts which are not infected and not immune at age a, $X(a)$ is the proportion of hosts which are infected and not immune at age a. Variables $Y_0(a)$ and $Y(a)$ are defined similarly but for cohorts –not infected and immune} and –infected and immune} respectively. The following system of ordinary differential equations defines the dynamics over age of this system:

$$\frac{dX_0}{da} = -\beta X_0 + \mu X + \gamma Y_0,$$

$$\frac{dX}{da} = \beta(1-\alpha)X_0 - (\mu+\beta\alpha)X + \gamma Y,$$

$$\frac{dY}{da} = \beta\alpha X_0 + \beta\alpha X - (\gamma+\mu)Y,$$

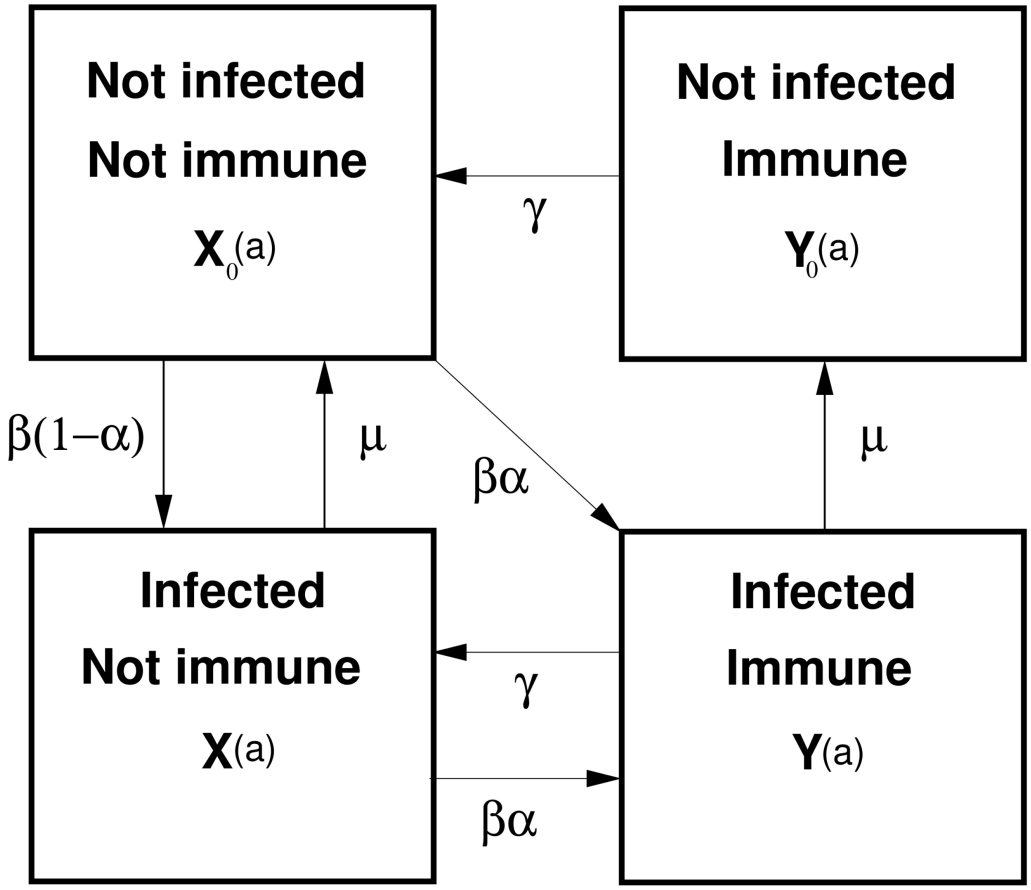

Figure 1. Transmission model for *E.multilocularis* in foxes. State variables are: $X_0(a)$, $X(a)$, $Y_0(a)$ and $Y(a)$, where $X_0(a)$ represents the proportion of hosts (foxes) which are not infected and not immune at age a, the other state variables are similarly defined. Parameter β denotes the infection pressure (force of infection), measured in insults (exposures) per year; α is the probability of immunity on exposure; γ is the rate of loss of host immunity; μ is the parasite death rate.
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$$\frac{dY_0}{da} = \mu Y - \gamma Y_0$$

with initial conditions: $X_0(0)=1$, $X(0)=0$, $Y_0(0)=0$ and $Y(0)=0$. Parameter β denotes infection pressure (force of infection - FOI), measured in insults (exposures) per year; α is the probability of immunity on exposure; γ is the duration of host immunity; μ is the parasite death rate. Note that to simplify the notation we have suppressed any explicit dependency of the parameters on age, e.g. $\beta(a)$ where FOI is dependent upon age, but such dependencies are considered during the model selection process making this potentially an inhomogeneous ODE system.

Model fitting and statistical analyses

The observed data comprise of randomly sampled binary observations each denoting whether a fox was infected (not infected). This gives a sampling model comprising of Bernoulli trials where the likelihood function for n observations is $\Pi_{i=1}^{n} p(a_i)^{I_i}(1-p(a_i))^{1-I_i}$, where a_i is the age of the ith fox in the data, I_i is an indicator variable where $I_i=1$ if the ith fox is infected and $I_i=0$ otherwise, and $p(a_i)=X(a_i)+Y(a_i)$ is the prevalence in foxes of age a_i. The ODE transmission model provides $p(a)$ which will generally be some unknown function of the epidemiological parameters of interest, $p(a)=f(\alpha,\beta,\gamma,\mu,a)$ where (Figure 1): α is the probability of immunity on exposure; β the force of infection (measured in insults per unit time); γ the rate of loss of immunity; and μ the parasite death rate. It is not necessary to know function f explicitly, all that is required is that for any given values of α,β,γ,μ, along with appropriate initial conditions for state variables X_0, X, Y_0, Y, an estimate for $p(a)$ for any suitable value of a can be computed. This is readily possible using standard numerical techniques for solving ODEs (e.g. [29]). The likelihood function ($\times$ parameter priors as we are using Bayesian inference) can therefore be evaluated, and thus the key unknown epidemiological parameters of interest such as β can be estimated from the study data —conditional on the chosen form of ODE model.

Gaussian distributed prior distributions for parameters β and γ were used, where these were each implemented within a log link function. For the probability parameter α, a logit link function was used, again with a Gaussian prior distribution. Highly diffuse priors were used for all parameters except μ, where these each had a mean of zero and standard deviation of $\sqrt{1000}$. In effect, this introduces no prior biological knowledge into the estimation of these parameters. For μ, a Gaussian prior (again on a log link) was used and chosen via expert opinion based on data presented in [17]. The latter study comprised of longitudinal observation of five foxes experimentally infected with *E. multilocularis*. The parasite burden in 80% (three of five) animals was very low at 90 days, suggesting an 80th percentile for the death rate of approximately ≤ 4 per year, in addition we consider that parasites in 50% of infected animals may survive to around 120 days (death rate ≤ 3 per year), with 2.5% possibly surviving beyond 150 days (death rate ≤ 2.4 per year). A Gaussian distribution on a log link with a mean of 1.2 and standard deviation of 0.2, gives quantiles for μ (on real scale) of approximately 2.24 (2.5%), 3.32 (50.0%) and 3.93 (80%) per year, which we choose as an informative prior for μ. In addition we also examine a wider, but still highly informative prior, with a mean of 1.3 and standard deviation of 0.3 which has corresponding quantiles of 2.04 (2.5%), 3.67 (50.0%) and 4.72 (80%) per year. Sensitivity to prior assumptions is a crucial aspect of Bayesian inference, so we also present modelling results which use the same highly diffuse (uninformative) prior for μ as for β and γ.

Bayesian model selection — used to identify an optimal ODE transmission model — was performed using the marginal likelihood goodness of fit metric. This is equivalent to comparing Bayes factors between two models when each has an equal a priori probability of being the preferred model. The marginal likelihood is generally more difficult to compute than other commonly used metrics, such as the Bayesian Information Criterion (BIC) or Deviance Information Criterion (DIC), but is the standard and preferred theoretical choice in Bayesian inference [26,30]. This metric allows Bayesian model selection to be interpreted as simply an extension of maximum likelihood model selection, where evidence (i.e. statistical support) for any given model is that obtained by multiplying the best fit likelihood by the "Occam factor", so-named as this metric has been shown to be conceptually consistent with Occam's Razor (as explained in [30]). The marginal likelihood was computed using Laplace approximations, a standard numerical technique in statistical inference [31,32]. These were also used to estimate posterior distributions for the epidemiological parameters. All numerics were implemented in R [33] using a number of well tested internal functions borrowed from the R abn library [34]. See Supporting Information Text S1 for technical details. An approximate guide for the size of differences in marginal likelihoods which may be considered notable is given in Table 2.1 page 27 in [26]. Using the terminology from [26], a difference of $0-2$ is suggested as weak support for the model with higher marginal likelihood, $2-6$ is support, $6-10$ is strong evidence and greater than 10 very strong evidence.

Results

We first present a brief exploration of the observed prevalence data by age. This is prudent as it may suggest refinements in the parametrization of the process models under consideration. Next we compare the goodness of fit of a range of models with different biological assumptions, for example whether the observed data support the presence of immunity, and if so, whether this is lifelong or transient. We then quantify the key epidemiological parameters in our chosen model, in particular the FOI, $\beta(a)$. Heterogeneity is then introduced into this model by allowing the force of infection to differ across one or more of the three different habitat types, where further model selection is used to identify a preferred heterogeneous model. Our results conclude with a comparison of FOI estimates across the different habitat zones.

Exploratory analyses by age

Exploratory analyses of the observed prevalence data is illustrated in Figure 2. Choosing a smoothing parameter of $f = 0.072$ in (lowess() in R) gives smoothed data which appear relatively consistent with the observed data in Figure 2 (a), and provides a more refined visualization of the data rather than in 30-day blocks. Figure 2 (a) and 2 (b) suggest that it may be appropriate to consider the inclusion of periodicity into one or more of the epidemiological parameters in our transmission model.This suggests that for our model to adequately capture the gross dynamic features of disease transmission we should consider both age independent FOI, $\beta(a) = \beta_0$, and also FOI parametrized as a function of age, $\beta(a) = g(a)$, with $g(a)$ as some polynomial or periodic function. It is clear from Figure 2 (c) that there appears very little identifiable dynamic structure after 36 months, which is perhaps unsurprising given this only comprises some 14% on observations, and thus very sparse sampling at these older ages.This is consistent with life expectancy estimates for foxes which suggest that only a small proportion of foxes survive beyond 2–3 years years in the wild [35]. As foxes aged less than three years present the vast majority of zoonotic risk, combined with foxes of older ages being sampled very sparsely in the data, subsequent analyses focus on foxes less than three years of age. For completeness some modelling results are also presented considering all ages. Figure 2 (d) shows the smoother applied to data of all ages.

Determining a parsimonious transmission model

A range of transmission models of increasing complexity were fitted to the observed data (Table 1) with separate results shown for the two informative priors for μ. See Supporting Information Text S2 for results using an uninformative prior for μ, and Supporting Information Text S3 for the equivalent of Table 1 but for the models fitted to data from foxes of all ages. Estimates of the posterior modes for all the parameters in models presented in Table 1 can be found in Supporting Information Text S4.

Evaluation of immunity

We commenced with a model comprising no immunity (Model 1-C), i.e. only state variables X_0 and X, and constant FOI. This was followed by similar models but where the FOI was parametrized as a linear (1-L), quadratic (1-Q) and periodic (1-P) function of age, with the latter using a sinusoidal forcing term as is commonly used for diseases with periodic transmission rates (e.g. measles [36]). The particular form of sinusoidal function used was $\log\{\beta(a)\} = \beta_0 + \beta_1 \sin\left\{2\pi\left(a - \frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$. A log link function ensures that all estimates of $\beta(a)$ are positive, and also avoids the potentially complex task of having to specifying a proper (i.e. integrates to unity) joint parameter prior for β_0, β_1 and a_s which would otherwise be required to ensure that the posterior distribution for $\beta(a)$ was positive. This parametric form of $\beta(a)$ has a period of one year, with (on a log scale) β_0 denoting the lifetime average (or baseline) FOI, β_1 the amplitude beyond the lifetime average. The term $\exp(a_s)/(1+\exp(a_s))$ is to allow, if

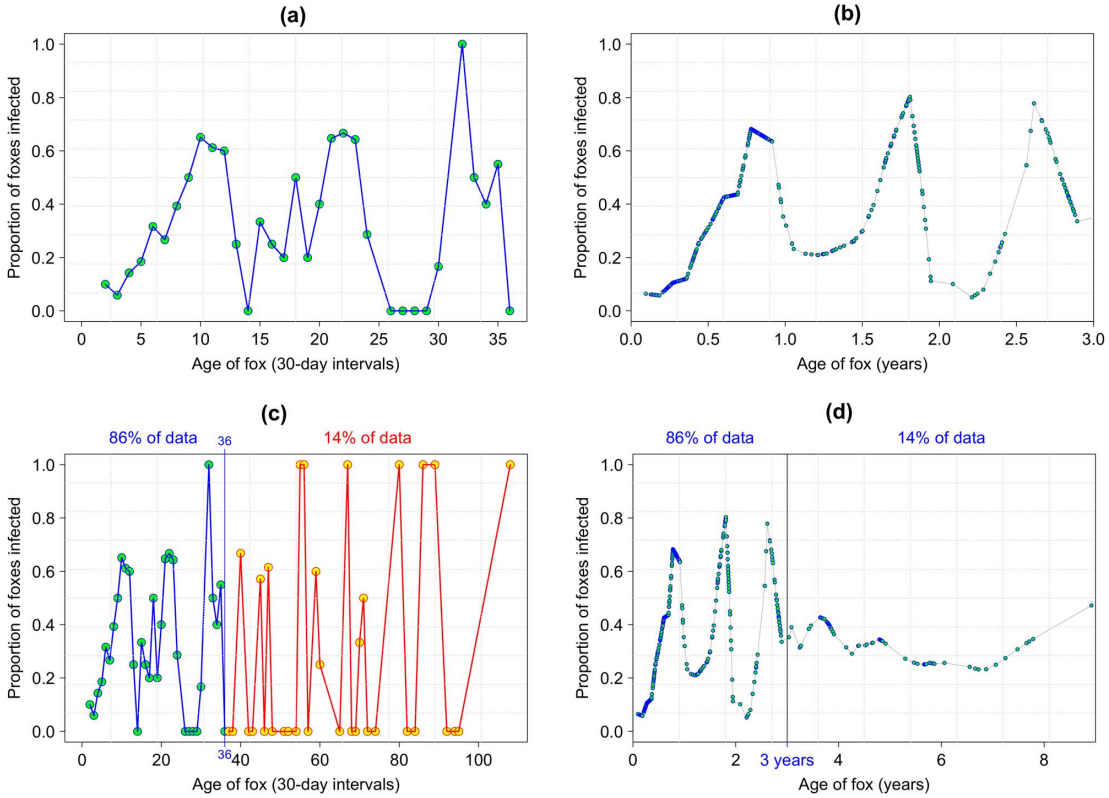

Figure 2. Exploratory analyses. Panel (a) shows observed prevalence across age groups of 30-days blocks up to age 36 months (where 1 month = 30 days). Panel (b) shows smoothed prevalence using a locally weighted regression smoother (lowess() in R) applied to the 0/1 observation for all individuals aged less than 3 years. Panel (c) shows observed prevalence across age groups of 30-days blocks for all ages (maximum 108 months where again one month = 30 days). Panel (d) shows the smoother applied to data of all ages.
doi:10.1371/journal.pntd.0002731.g002

Table 1. Model goodness of fits.

Model	Description	Prior for μ	Log marginal likelihood
1-C	no immunity ($\alpha=0$) Constant FOI: $\log\beta(a)=\beta_0$	$N(1.2,0.2)$ $N(1.3,0.3)$	-305.3 ($\Delta_{ML}=28.2$) -304.3 ($\Delta_{ML}=26.2$)
1-L	no immunity ($\alpha=0$) Linear FOI: $\log\beta(a)=\beta_0+\beta_1 a$	$N(1.2,0.2)$ $N(1.3,0.3)$	-309.3 ($\Delta_{ML}=36.2$) -308.9 ($\Delta_{ML}=35.4$)
1-Q	no immunity ($\alpha=0$) Quadratic FOI: $\log\beta(a)=\beta_0+\beta_1 a+\beta_2 a^2$	$N(1.2,0.2)$ $N(1.3,0.3)$	-308.1 ($\Delta_{ML}=33.8$) -308.3 ($\Delta_{ML}=34.2$)
1-P	no immunity ($\alpha=0$) Periodic FOI: $\log\{\beta(a)\}=\beta_0+\beta_1\sin\left\{2\pi\left(a-\frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(1.2,0.2)$ $N(1.3,0.3)$	-291.3 ($\Delta_{ML}=0.2$) -291.2 ($\Delta_{ML}=0.0$)
2	lifelong immunity ($\gamma=0$) periodic FOI: $\log\{\beta(a)\}=\beta_0+\beta_1\sin\left\{2\pi\left(a-\frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(1.2,0.2)$ $N(1.3,0.3)$	-294.3 ($\Delta_{ML}=6.2$) -294.6 ($\Delta_{ML}=6.8$)
3	transient immunity ($\gamma\neq0$) periodic FOI: $\log\{\beta(a)\}=\beta_0+\beta_1\sin\left\{2\pi\left(a-\frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(1.2,0.2)$ $N(1.3,0.3)$	-294.2 ($\Delta_{ML}=6.0$) -296.0 ($\Delta_{ML}=9.6$)

All parameters other than μ have diffuse priors as given in the text. The Δ_{ML} denotes twice the difference between the best log marginal likelihood and each of the other models.
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necessary, a time shift compared with the standard sinusoidal function. A logit link function is used here as we are only interested in time shifts in the interval [0,1]. Parameters β_0,β_1 and a_s each have diffuse Gaussian priors with means of zero and standard deviations of $\sqrt{1000}$.

From Table 1 is it clear that periodic infection pressure is strongly supported over the other forms. Retaining periodic infection pressure, we next consider models with a more complex cohort structure comprising of all four state variables $\{X_0,X,Y,Y_0\}$, allowing for the presence of lifelong immunity (Model 2), and transient immunity (Model 3 and the "full" model in Figure 1). It is again apparent from Table 1 that the observed data are less supportive of these two more complex models, and hence there is little evidence in the data for the presence of immunity.

Based purely on the goodness of fit results in Table 1 our preferred model is Model 1-P. The next more complex best fitting model was Model 2. These two models cross a rather large biological divide — no immunity verses lifelong immunity. To provide additional empirical justification for choosing Model 1-P over Model 2 we briefly examine the magnitude of the parameters in the latter model using the posterior modes (which are estimated as part of the marginal likelihood computation). In Model 2, using the prior for μ with mean of 1.2, we have a logit for α of -5.3 giving an approximate probability of becoming immune per exposure of 0.005. Posterior mode estimates for the FOI in this model, $\beta(a)$, gives an (approximate) average lifetime number of exposures, $\exp(\beta_0)$, of ≈ 2 per year. Based on the observed prevalence data, then suppose that 86% of animals have a lifetime of at most three years and the remaining 14% live for a full nine years. Then, in a population of 100 animals these parameters give a total of 768 exposures for all animals over their entire lifetime. For $\alpha = 0.005$ this then gives, on average, at most only four animals becoming immune during the entire lifetime of the population. This is a very fine scale population change, and it is therefore of little surprise that, statistically, the empirical data are not supportive of the presence of immunity.

Quantification of force of infection

Having arrived at a preferred transmission model we now use this to provide the first of our main results: quantification of the FOI, i.e. $\beta(a)$. Of most interest here are the baseline and amplitude parameters β_0 and β_1, specifically we wish to estimate the joint marginal posterior distribution for these two parameters and then examine the range of values for the FOI which arise when (β_0,β_1) are within their joint 95% posterior confidence interval (to account for sampling uncertainty). It would be possible to consider a joint density comprising of all three parameters in $\beta(a)$; β_0,β_1,a_s. It is, however, difficult to visualize such a density (with four dimensions - three parameters plus the density estimate), and as epidemiological interest is focused on (β_0,β_1) we therefore marginalize out a_s and μ giving a joint posterior density for (β_0,β_1). Note that this distribution, therefore, also incorporates the statistical uncertainty in a_s and μ (i.e. the latter are not simply fixed at constant values).

Before computing the joint marginal density for (β_0,β_1) we first summarize β_0, β_1, μ and a_s through their marginal posterior 95% confidence intervals (Supporting Information Text S5 provides full marginal posterior densities). Using the informative prior for μ with mean = 1.2 and sd = 0.2 gives (on the real scale) $\beta_0 = (1.32,2.79)$, $\beta_1 = (2.27,4.55)$, $a_s = (0.35,0.48)$ and $\mu = (2.38,4.82)$, with approximate medians of $\beta_0 = 1.92$, $\beta_1 = 3.14$, $a_s = 0.42$; and $\mu = 3.36$. The corresponding estimates when using the informative prior for μ with mean = 1.3 and sd = 0.3 are $\beta_0 = (1.34,3.34)$, $\beta_1 = (2.29,4.55)$, $a_s = (0.35,0.49)$ and $\mu = (2.30,6.14)$, with approximate medians of $\beta_0 = 2.087$, $\beta_1 = 3.17$, $a_s = 0.42$; and $\mu = 3.74$. Using the diffuse prior for μ gives $\beta_0 = (1.16,4.19)$, $\beta_1 = (2.33,4.51)$, $a_s = (0.38,0.54)$ and $\mu = (1.69,8.25)$, with approximate medians of $\beta_0 = 2.24$, $\beta_1 = 3.20$, $a_s = 0.50$; and $\mu = 3.98$.

A contour plot of the joint marginal posterior density for (β_0,β_1), Figure 3 panel a, clearly shows strong dependency between β_0 and β_1 — when one is lower the other is higher and vice-versa. This demonstrates why it is more intuitively reasonably to consider these parameters jointly. To visualize the statistical uncertainly in our estimate of FOI over age we choose two points $pt_{1,95\%} = (\beta_0,\beta_1)$ and $pt_{2,95\%} = (\beta_0,\beta_1)$, which lie on the contour defining the 95% region for this two-dimensional density. We then solve the ODE model for these sets of parameter estimates (the other two parameters are set to their modal values). These two "extreme" sets of parameters provide an approximate 95% confidence interval for the mean force of infection over age (Figure 3 panel b), and similarly the mean prevalence (Figure 3 panel c). We estimate the (mean) minimum FOI during an annual population cycle as 0.27 to 1.27 insults (with 95% confidence), and rising to a maximum of between 6.87 and 7.05 insults (with 95% confidence).

Comparison between urban and rural habitats

The summary statistics suggest that there may be a difference between the prevalence of *E.multilocularis* in populations of foxes within the different habitat types. To provide a measure of statistical rigour to these observations we fit Model 1-P to these data, where now heterogeneity is introduced into $\beta(a)$ to allow the force of infection to vary across each of the different zones. If the inclusion of such heterogeneity improves the model goodness of fit then that provides formal statistical evidence of a different in FOI between habitats.

We consider two versions of Model 1-P, Model 1-P^0 and Model 1-P^{01}. The first allows the baseline force of infection, β_0, to vary with zone and assumes the amplitude β_1 is homogeneous across all zones. The second model allows both β_0 and β_1 to vary within each habitat zone. For simplicity, the period shift a_s and parasite death rate μ are assumed homogeneous over all three zones. Model 1-P^0 has a goodness of fit of -285.4, with Model 1-P^{01} having -292.6. This is strong evidence that: i) there is a difference in baseline force of infection between different habitat zones; ii) there is no evidence of any difference in periodic amplitude between the different habitats. We use, therefore, Model 1-P^0 to quantify differences in FOI across habitat.

Following a similar approach as for our analyses of Model 1-P, we derive approximate confidence intervals for the force of infection using the joint marginal posterior densities for β_0 and β_1, where this time we have three, two dimensional distributions, (β_0^U,β_1), (β_0^B,β_1), (β_0^P,β_1) for U urban, B border and P periurban. First we summarize $\beta_0^U,\beta_0^B,\beta_0^P,\beta_1,\mu$ and a_s through their marginal posterior 95% confidence intervals (Supporting Information Text S6 provides full marginals posterior densities). Using the informative prior for μ with mean = 1.2 and sd = 0.2 gives (on the real scale) $\beta_0^U = (0.45,1.27)$, $\beta_0^B = (1.20,2.94)$, $\beta_0^P = (2.42,6.18)$, $\beta_1 = (1.48,3.2)$, $a_s = (0.29,0.47)$ and $\mu = (2.29,4.50)$, with approximate medians of $\beta_0^U = 0.79$, $\beta_0^B = 1.87$, $\beta_0^P = 3.79$, $\beta_1 = 2.13$, $a_s = 0.38$ and $\mu = 3.14$. It is clear that the marginal densities in the urban and periurban habitats do not overlap at the 5% significance level. Supporting Information Text S7 provides a comparison of the modal estimates of prevalence over age in each of the three habitat types.

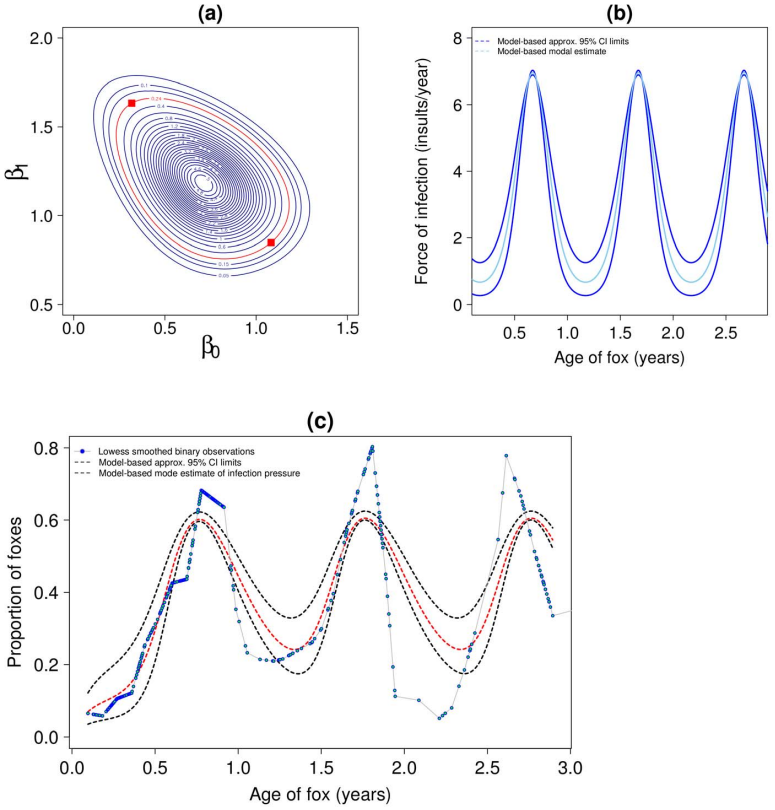

Figure 3. Transmission Model 1-P. Panel (a): joint marginal posterior density for (β_0,β_1) on log scale. The red contour is the 95% limit and the two points marked are those used to produce approx. 95% confidence intervals in panels b and c. Panel (b): dynamics of force of infection by age, 95% CI is for the mean force of infection at age a. Panel (c): Smoothed observed prevalence and prevalence predicted by Model 1-P, 95% CI are for the mean prevalence at age a. All results use the informative prior for μ with mean = 1.2 and sd = 0.2.
doi:10.1371/journal.pntd.0002731.g003

Finally we consider the statistical uncertainty in our FOI estimates over age within each habitat type. Figure 4 panel a is similar to Figure 3 panel a and shows the joint marginal posterior densities for (β_0^U,β_1), (β_0^B,β_1), (β_0^P,β_1). As for the one-dimensional marginal estimates of β_0 in each habitat, it is very clear that the FOI baseline is statistically different between the urban and periurban zones i.e. the 95% contours do not overlap. The FOI in the border zone is indistinguishable from that in either the periurban or rural zones. We repeat the same approach to estimate approximate 95% confidence intervals for the FOI within each habitat as for the homogeneous habitat model (Model 1-P), this is shown in Figure 4 panel b. These uncertainty limits are clearly rather more approximate here than for those in Model 1-P — as can be seen by the fact that the urban and periurban trajectories overlap slightly, while they are clearly very distinct at the 95% contours in Figure 4 panel a. The limits for the border habitat also cross each other. This behavior is not entirely unexpected in that we are collapsing a six dimensional posterior probability distribution (comprising of all the parameters in Model 1-P^0) into effectively only two dimensions. This gives joint statistical estimates which are far more manageable, but as we see here, does makes the resulting confidence limit estimates rather approximate. We estimate with approximate 95% confidence that the (mean) minimum FOI during an annual cycle in the urban habitat is 0.1 to 0.8 insults, rising to a maximum of between 1.6 and 2.0 insults. For the periurban habitat we have minimum and maximum force of infections of 0.7 to 3.9 insults and 9.35 to 9.7 insults respectively. Despite these minor statistical discrepancies in relation to the differing comparisons of confidence limits, the overall result is very clear: there is a large difference in FOI during annual cycles in the urban and periurban habitats.

Discussion

The FOI is a key parameter in models estimating the effectiveness and cost effectiveness of infectious disease prevention [37]. Using a simple —and empirically justified — mathematical model we have estimated the force of *E. multilocularis* infection in a fox population in Switzerland, and shown how much it quantitatively varies with season and geography, i.e. through time and across space.

There have been a number of trials aimed at reducing the prevalence of infection in foxes by distributing baits containing the anthelmintic praziquantel. Several studies, in Switzerland and in Germany, with baiting intervals of 12 times per year, resulted in a substantive decline in the numbers of foxes infected (reviewed in

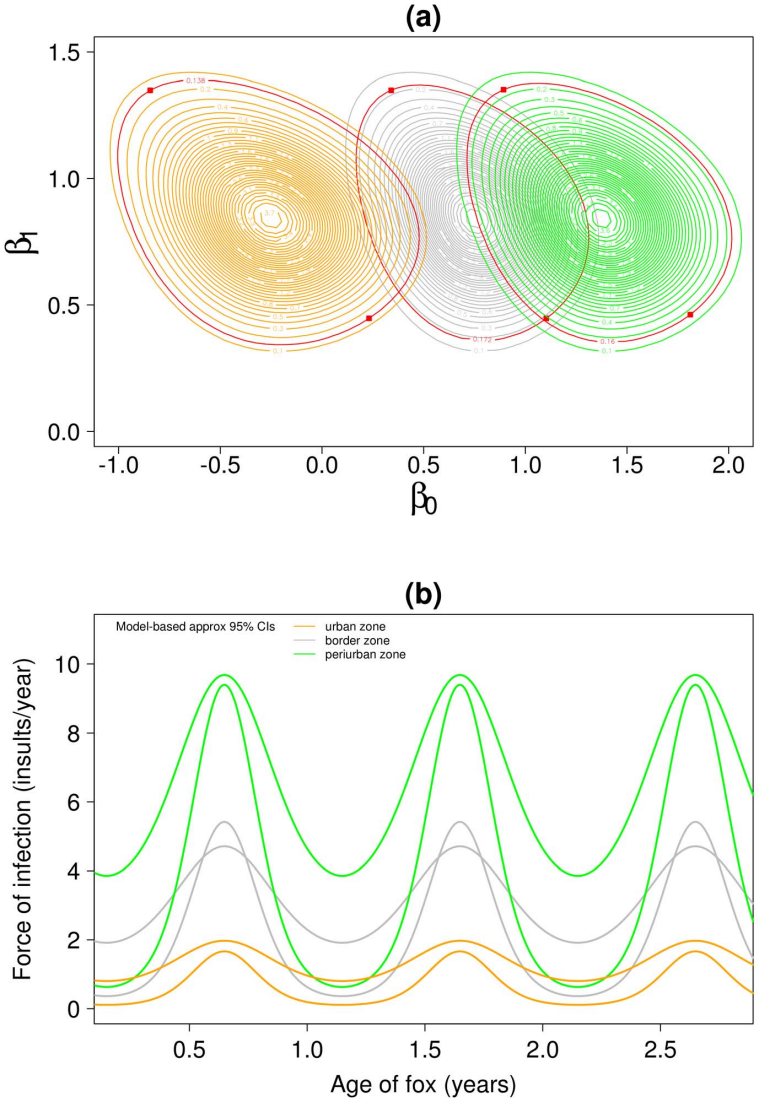

Figure 4. Heterogeneous habitat transmission Model 1-P^{o}. Panel (a): joint marginal posterior densities for (β_0^U,β_1), (β_0^B,β_1), (β_0^P,β_1) on log scale. The red contour is the 95% limit and the two points marked are those used to produce approx. 95% confidence intervals in panel b. Panel (b): dynamics of force of infection by age, approx 95% CI is for the mean force of infection at age a (see main text for explanation of why these lines cross). All results use the informative prior for μ with mean = 1.2 and sd = 0.2.
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[22,38,39]). These studies typically resulted in a decrease in prevalence from 35% and 67% to between 1% and 6%. Provided most foxes are treated, this would be expected as the baiting interval is similar to the prepatent period of *E. multilocularis* in foxes and hence it should prevent transmission. Other baiting campaigns have used lower frequencies and have had variable results. For example in Germany a baiting frequency of 5 times per year resulted in a decrease in the prevalence in foxes of 32% (95% CIs 16–52) to 4% (95% CIs 2–7). Other studies with less frequent baiting intervals have not shown such a clear reduction. Our estimates and modelling methodology for computing the pre-intervention baseline FOI provides a rigorous framework which can be used to optimize baiting intervals, in order to trade off the need to reduce infection in foxes, and thus the potential for zoonotic transmission, and the cost of implementing such intervention programmes. Based on Swiss data we estimate that there is a high infection pressure in the winter months for non urban foxes of close to 10 infections per year (i.e. greater than 1 per month), baiting at monthly intervals would therefore be required. This conclusion is in accordance with the results of an epidemiological study on the intermediate hosts which showed most rodents become infected during the winter [23]. However, in

the summer when the FOI is lowered to between 0.7 to 3.9 insults per year, then decreasing the baiting frequency to once every three months would be more appropriate. In addition, baiting frequency, at least in theory, could be further reduced in urban habitats where the FOI is between 0.1–0.8 and 1.6–2.0 insults per year. However in practice, this would be a challenge in Zürich as the spatial separation of such zones is as little as 500 meters. A decreased cost of baiting foxes increases the cost benefit as a similar reduction in the numbers of human AE cases would be expected to be achieved as earlier suggested [15] based on epidemiological data [23,24]. Theoretical models [40,41], have also suggested seasonal transmission of *E. multilocularis* in Japan. However, our model is also challenged with field data, where as the conclusions of previous models are based on simulations. In addition, our model does not depend upon parameters from the intermediate host and therefore should be applicable for FOI calcualtions in any area where suitable prevalence data from foxes is available.

Our estimates of FOI are dependent on the estimate of the life expectancy of the infection in the definitive host. Experimental infections of foxes indicate that parasites can survive in foxes beyond 90 days [17], although most parasites are lost earlier. This model is based on the presence or absence of parasites, with even a single parasite being found in a fox defining the fox as infected. Therefore an estimated life expectancy of 120 days was used in the model as being a reasonable period extrapolating from the data of [17]. By which half of foxes might be estimated to be free of parasites. If the life expectancy is less then the FOI will be higher than reported here. The corollary is also true. A longer life expectancy would result in a lower FOI. It is possible that low worm burdens in foxes could persist for some considerable time as all foxes in the experimental study by Kapel and others [17] remained infected at 90 days, albeit with low burdens. However, if this were the case, decreasing baiting frequency in the summer months and in urban areas, as suggested would still be effective in lowering the parasite biomass, as the numbers of infections per year would be lower than calculated here. However, as infection is highly overdispersed only a few infected foxes will be responsible for most of the transmission. Using a non zero threshold worm burden for foxes that are relevant to transmission could give important information with regard to the FOI in heavily infected foxes. An alternative approach, in a future study, using abundance data may help clarify this issue. An obvious related key question is quantifying the transmission probability from environmental contamination, e.g. via the distribution of fox faeces, to human infection.

To finish, a brief comment on the basic reproduction ratio (R_0), arguably the most important epidemiological parameter in any disease system, although it is not without its critics [42]. Robust estimation of R_0 is often difficult, especially with parasites with complex life cycles. Roberts [43] described how R_0 could be estimated if prevalence data from foxes and small mammal intermediate hosts were available together, along with a number of assumptions regarding various transmission parameters. However, when it is difficult to estimate R_0, estimates of FOI become highly relevant [37]. We have shown that with a relatively simple transmission model empirically justified from study data, an estimate of the FOI can be made, and how this can be practically applied for optimizing the interval of baiting to lower the prevalence of *E. multilocularis* in foxes.

Supporting Information

Data S1 File containing original data.
(XLS)

Text S1 Estimating the marginal likelihood.
(PDF)

Text S2 Results using an uniformative prior for μ.
(PDF)

Text S3 Modeling results for foxes of all ages.
(PDF)

Text S4 Estimates of the posterior modes for all the parameters in models presented in Table 1.
(PDF)

Text S5 Full marginal posterior densities for model 1-P for the parameters β_0, β_1, a_s and μ using the informative prior μ with mean = 1.2 and s.d. = 0.2.
(PDF)

Text S6 Full marginal Posterior densities for model $1-P_0$ for the parameters β_0, β_1, a_s and μ using the informative prior μ with mean = 1.2 and s.d. = 0.2.
(PDF)

Text S7 Model prevalence estimates by habitat using model 1-P^0.
(PDF)

Author Contributions

Conceived and designed the experiments: FIL BOA PRT. Performed the experiments: FIL BOA DH PD PRT. Analyzed the data: FIL BOA PRT. Contributed reagents/materials/analysis tools: FIL BOA DH PD PRT. Wrote the paper: FIL BOA DH PD PRT. Collection of data: DH PD.

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Paper 3

Mathematical modelling of *Echinococcus multilocularis* abundance in foxes in Zurich, Switzerland

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Mathematical modelling of *Echinococcus multilocularis* abundance in foxes in Zurich, Switzerland

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Abstract

Background: In Europe, the red fox (*Vulpes vulpes*) is the main definitive host of *Echinococcus multilocularis*, the aetiological agent of a severe disease in humans called alveolar echinococcosis. The distribution of this zoonotic parasite among the fox population is remarkably aggregated with few heavily infected animals harbouring much of the parasite burdens and being responsible for most of the environmental parasitic egg contamination. Important research questions explored were: (i) spatial differences in parasite infection pressure related to the level of urbanization; (ii) temporal differences in parasite infection pressure in relation to time of the year; (iii) is herd immunity or an age-dependent infection pressure responsible for the observed parasite abundance; (iv) assuming *E. multilocularis* infection is a clumped process, how many parasites results from a regular infection insult.

Methods: By developing and comparing different transmission models we characterised the spatio-temporal variation of the infection pressure, in terms of numbers of parasites that foxes acquired after exposure per unit time, in foxes in Zurich (Switzerland). These included the variations in infection pressure with age of fox and season and the possible regulating effect of herd immunity on parasite abundance.

Results: The model fitting best to the observed data supported the existence of spatial and seasonal differences in infection pressure and the absence of parasite-induced host immunity. The periodic infection pressure had different amplitudes across urbanization zones with higher peaks during autumn and winter. In addition, the model indicated the existence of variations in infection pressure among age groups in foxes from the periurban zone.

Conclusions: These heterogeneities in infection exposure have strong implications for the implementation of targeted control interventions to lower the intensity of environmental contamination with parasite eggs and, ultimately, the infection risk to humans.

Keywords: *Echinococcus multilocularis*, Alveolar echinococcosis, Epidemiology, Transmission, Mathematical modelling

Background

Echinococcus multilocularis is a zoonotic cestode present in large parts of the northern hemisphere. The parasite is sustained by a wildlife cycle with carnivores (mainly foxes) as definitive hosts and small mammals (mainly rodents) as intermediate hosts [1]. However, domestic dogs are believed to be an infection source for humans in Asia [2, 3]. Humans are accidental hosts that become infected through the ingestion of parasitic eggs excreted through the faeces of the infected canids [1]. The metacestode stage of this tapeworm causes chronic life-threatening alveolar echinococcosis (AE), which can have a high economic impact in highly endemic resource-poor settings [4]. The geographic distribution of *E. multilocularis* seems to be expanding and it is considered an emerging/re-emerging pathogen in many countries [5–8]. In Europe, high prevalences (23.9–57.3%) of *E. multilocularis* have been frequently reported in the red fox population (*Vulpes vulpes*) [9–11], which is increasingly colonising urban areas [12, 13]. In Zurich (Switzerland), the abundant availability of anthropogenic food seems to have contributed to the gradual increase of the urban fox population [14].

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Moreover, the establishment of an *E. multilocularis* transmission cycle in the urban and periphery of Zurich is well documented [11, 15–17] as conditions appear to sustain high densities of foxes that support an active parasite life-cycle. These findings, along with the increasing incidence of human AE [18] have raised public health concerns and the demand to implement disease control strategies [15, 19].

Variations between individuals in their exposure and susceptibility to parasite infective stages result in the aggregated distribution of parasites within their hosts [20]. The distribution of *E. multilocularis* in foxes is also characteristically aggregated with most animals carrying low numbers of parasites whereas a few harbour thousands of them. The risk of developing human AE depends, among other factors, on the amount of infective eggs found in the environment and their accessibility to humans [21]. Due to the parasite aggregation, the degree of egg contamination in the environment depends greatly on a few highly infected animals [11, 15, 22]. However, as eggs can survive in the environment for some time, there may also be some contribution from less heavily infected foxes. Information on prevalence in foxes has been often used to characterize the infection risk for AE as its estimation is more reliable and straightforward than other epidemiological parameters [23]. However, there is not a clear correspondence between prevalence rates and parasite abundance in the fox population [24]. Hence, there are major limitations when using prevalence in foxes to describe the epidemiology of *E. multilocularis* infection [11]. The determination of parasite abundance in animal hosts can provide valuable information to optimize parasite control strategies. For instance, if there is evidence of spatial heterogeneities in *E. multilocularis* infection pressure, anthelmintic baits can be distributed in areas where superinfected animals are predicted to be in order to reduce more efficiently the environmental contamination of eggs and ultimately, human infection. A key epidemiological parameter to predict parasite abundance in the animal host is the infection pressure. The parasite burden in the definitive host depends on the number of infectious stages encountered per infection insult, meaning the number of viable protoscoleces contained in the hydatid cysts that the intermediate host carries. This study complements the results reported on the force of infection by a study on the mathematical modelling of *E. multilocularis* infection in foxes in Zurich [25]. There, the force of infection is defined as the number of exposures per unit time regardless of the quantity of parasites to which a fox is exposed [25].

The infection pressure cannot be estimated through direct observation; hence, we use mathematical models that allow inference on processes relevant to transmission as well as their quantification, in conjunction with field data. Besides the specific research question we want to address and the identification and incorporation of the epidemiological knowledge available, the selection of an appropriate model will depend on its ability to represent the available field data. The data for the present study consisted of parasite counts found in necropsied foxes collected in three different spatial zones within the municipality of Zurich [17]. Several studies have been carried out on *E. multilocularis* transmission in foxes in Switzerland providing an extensive prior knowledge for model construction and hypothesis formulation. Previous studies of *E. multilocularis* in Switzerland have shown that transmission dynamics in animal hosts are influenced by multiple interrelated factors that contribute to its spread [11, 17, 26–28]. Decreasing parasite prevalences along with the increasing level of urbanization have been reported in foxes in the two largest cities of Switzerland [11, 27, 28]. Special attention was brought to the intermediate areas between the rural and urban habitats where the proportion of *E. multilocularis* coproantigen-positive fox faeces was higher compared to the urban area [16]. These areas are believed to be heavily contaminated by infective eggs, and thus may represent *hot-spots* for human infection [15]. In addition, there is evidence of seasonal variation in parasite abundance in Swiss foxes, which has been found to be related with the age of the host [11, 17, 26]. In addition, juvenile foxes of less than one-year-old have frequently been reported bearing higher infection rates and parasite burdens [11, 17, 26, 27, 29]. The study quantifying the force of infection in *E. multilocularis* in foxes in Zurich, defined as the number of fox exposures to parasite infection (insults) per unit time, reported spatial and seasonal variations in incidence of exposure [25]. However, it did not address parasite abundance, which is important for the transmission dynamics. Here, we adapted existing transmission models describing the number of parasites depending on host age [30, 31] to estimate the spatio-temporal variation of the infection pressure. We aim to address further specific research questions: (i) are there spatial differences in parasite infection pressure related to the level of urbanization; (ii) are there temporal differences in parasite infection pressure in relation to time of the year; (iii) is herd immunity or an age-dependent infection pressure responsible for the observed parasite abundance; (iv) assuming *E. multilocularis* infection is a clumped process, how many parasites results from a regular infection insult.

Methods

Study data

The data used for this study was sourced from the necropsies of red foxes collected from January 1996 to April 2000 within the political community of Zurich as part of the Integrated Fox Project and before the implementation of an anthelmintic baiting study [11, 17]. The age

of each fox was determined in years through dental examination [32] assuming all cubs were born on the first of April, as described previously [33]. In this study, we used the dates when the foxes were collected to estimate the approximate age in days of each fox. Foxes less than 1-year-old were classified as juveniles [34]. Each animal was further classified as coming from the periurban, border or urban zone, depending on where it was collected. The characteristics of each spatial zone have already been described in detail by Hegglin et al. [17]. The periurban zone refers to the external ring delimiting the city of Zurich and which mainly comprises a green belt of forests, fields, pastures, and meadows. The border area refers to the intermediate ring that contains residential areas, allotment gardens, cemeteries, sports fields, and public parks. The urban area refers to the center of the city encompassing much of the built-up zone.

For the analysis, we used *E. multilocularis* intestinal counts from 531 foxes aged up to 9 years old. Thus, the parasite biomass is the total number of parasites recovered from the foxes. The median age was less than 1-year-old in all zones. The group had an overall prevalence of 41.4% and a median abundance of 0 parasites (95% central range 0–10,488 parasites). All the data is provided in Additional file 1.

Age-based abundance model

The association between parasite burden and age in foxes [11, 26, 27] suggested the use of an age-stratified SIR model originally developed by Roberts et al. [30]. It stratifies the host population into compartments that represent their infection and immune status at a particular age and the transition between states can be described by a set of ordinary differential equations. A schematic representation of the model is given in Fig. 1.

The model describes the variation in the proportion of animals susceptible to infection (equation 1) and the change of parasite abundance with respect to the host's age t (equation 2).

$$\frac{dS}{dt} = \gamma - (\gamma + ah)S \quad (1)$$

$$\frac{dM}{dt} = hS - \mu M \quad (2)$$

where S is the proportion of susceptibles, t is the age of the host, γ is the rate of loss of immunity to parasites by foxes, a is the rate of acquisition of immunity, h is the infection pressure in number of parasites per year, M is the parasite abundance and μ is the parasite death rate ($1/\mu$ is the parasite life expectancy). The infection pressure, in the present report, is defined as the number of adult worms that would develop in the definitive host after parasite exposure in the absence of density-dependent constraints.

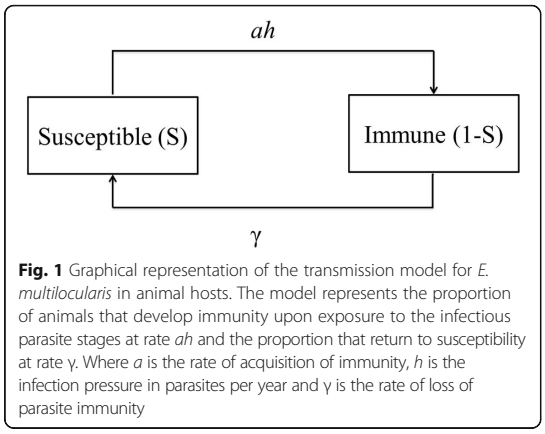

Fig. 1 Graphical representation of the transmission model for *E. multilocularis* in animal hosts. The model represents the proportion of animals that develop immunity upon exposure to the infectious parasite stages at rate *ah* and the proportion that return to susceptibility at rate γ. Where *a* is the rate of acquisition of immunity, *h* is the infection pressure in parasites per year and γ is the rate of loss of parasite immunity

By adapting these equations, we attempt to answer our questions on *E. multilocularis* infection pressure and build models describing different plausible scenarios that might explain the parasite abundance observed in the foxes. As a result, a series of models assessing the existence of spatio-temporal and age-dependent variations in infection pressure were developed. The model parameters and descriptions are summarized in Tables 1 and 3, respectively.

Models assessing spatial differences in the infection pressure

The study area was divided into three spatial zones, periurban, border, and central urban covering 20%, 41%, and 39% of the study area respectively. Three different scenarios were considered: (i) the study area comprised just one spatial zone; (ii) the study area comprised two different spatial zones, the periurban and the suburban which includes the border and urban zones and; (iii) the study area comprised three different spatial zones, periurban, border and urban. The border and urban area were merged

Table 1 Description of the abundance model parameters for *E. multilocularis* in foxes in Zurich

Parameter	Description
β_0	Baseline number of parasites of the infection pressure
β_p	Amplitude of the infection pressure in the periurban zone
β_b	Amplitude of the infection pressure in the border zone
β_u	Amplitude of the infection pressure in the urban zone
φ_p	Decrease parasite rate in the periurban zone
a	Rate of acquisition of immunity on exposure
γ	Rate of loss of immunity
μ	Parasite death rate

into one zone in the second scenario to consider the possibility of no spatial differences in infection pressure between both areas, as they are quite alike. These scenarios for different spatial zones were analyzed by using either 1 model with a single value for the infection pressure h, 2 different values for h for 2 zones and 3 values of h for 3 zones (in equations 1–4). Likewise, it was analyzed if there were potentially 1, 2, or 3 baseline infection pressure β_0 between the zones (see equations 3 and 4)

Models assessing time-dependent infection pressure

The evaluation of the time dependence of the infection pressure using host age as proxy for time resulted in three different functions (equation 3), where β_0 represented the baseline number of parasites in a year and β the amplitude by which this baseline could vary according to a linear or periodic relationship. The models accounted for different baseline infection pressures and amplitudes for each of the three urbanization zones: periurban (β_p), border (β_b) and urban (β_u). A log link function was implemented to ensure positive estimates of β_0 and β, as previously described [25].

$$\begin{aligned} &\text{Constant infection pressure}: \ ln\{h(t)\} = \beta_0 \\ &\text{Decrease in infection pressure}: \ ln\{h(t)\} = \beta_0 - \beta t \\ &\text{Periodic infiction pressure}: \ ln\{h(t)\} = \beta_0 - \beta sin(2\pi t) \end{aligned} \tag{3}$$

where the infection pressure (h) at age t is given by the amplitude (β) by which the baseline number of parasites (β_0) varies, or does not, in a year.

Models assessing age-dependent infection pressure

The models that assume the existence of an age-dependent infection pressure include a parameter (ϕ) representing the reduction in the number of parasites that foxes acquired after exposure which is proportional to the increase of host age. Thus, the infection pressure at age t where there is both periodic infection pressure and a decrease with age is given by equation 4:

$$\begin{aligned} &\text{Periodic with age-decrease in infection pressure}: \\ &ln\{h(t)\} = \beta_0 - \beta sin(2\pi t) - \phi t \end{aligned} \tag{4}$$

where h is the infection pressure in number of parasites per year, β_0 is the baseline number of parasites in a year, β is the amplitude by which this baseline varies periodically and ϕ is the decrease in the number of parasites related with fox age.

Model fitting

Echinococcus multilocularis follows a highly aggregated distribution within the animal hosts, thus, we used the negative binomial likelihood function to fit age-based abundance models to the observed data (equation 5).

$$\Pr(Z(t) = s) = \frac{\Gamma(k+s)}{\Gamma(k)\ s!}\left(\frac{M}{k+M}\right)^s\left(\frac{k}{k+M}\right)^k \tag{5}$$

where the probability of the number of parasites (s) for each sample (Z) at age (t) is given by the mean number of parasites (M) predicted by the model, where Γ represents the gamma distribution and k is the negative binomial constant of aggregation. The values for the aggregation constants for each spatial zone were estimated from the observed data using the *glm.nb* function from the MASS package in R [35]. In addition, we assumed a common negative binomial constant for all age groups, as it has been previously reported [31]. We explored variable aggregation between the zones by assigning different values of k to each zone.

The life expectancy of the parasite ($1/\mu$) was estimated from the model fit, allowing μ to be data driven. This was compared to an estimate of μ of 8.6 from the data presented in Kapel et al. [36]. Equations (1) and (2) including any variation in h over time, as described by equation (3), were numerically integrated using the ode function in the deSolve package in R [37].

Based on this probability model, a likelihood function was computed stating the probability to observe the data, given the model. The transformed, negative log-likelihood (NLL) function was minimized using the *optim* function of the statistical package in R [38]. All R code is provided in Additional file 2.

For model comparison and selection we followed the method described in Rüegg et al. [39]. The NLL of each competing model was plotted against the number of parameters. This method provided a visual aid to identify the best fitting models for each number of parameters. The selected models were then compared in pairs in increasing order of complexity, starting with the simplest model (M1). The difference of NLL between each pair of models was tested against an empirical probability distribution of the null hypothesis that the simpler model provides a better fit to the data. To this end, 500 populations were simulated from the simpler model. For each population the two competing models were fitted and the difference in NLL was computed. The NLL difference estimated from the data was then compared to the 95%-quantile of this distribution. Therefore, the more complex model would give a better fit just by chance in less than 5% of the cases ($\alpha = 0.05$).

Parameter estimation

Key epidemiological parameters were quantified from the best fitting model and confidence intervals were

estimated by bootstrapping. We generated 1,000 data sets and estimated the parameter values by resampling with replacement from the originally data set. That is creating 1,000 data sets, each of 531 data points being the size of the original sample of data. For the confidence interval, we reported the 2.5th and 97.5th percentiles of the bootstrap samples. For the confidence bands of the most parsimonious model, these 1,000 data sets were used to generate predicted abundances at each time point to then report their 2.5^{th} and 97.5^{th} percentiles.

Number of parasites per infectious insult

Using the results of the model quantifying the force of infection with the same data set [25], we estimated the numbers of parasites per infectious insult acquired by foxes in the periurban and urban zone at times of highest and lowest infection pressure, by using the simple equation:

$$Number\ of\ parasites\ per\ infectious\ insult = \frac{Infection\ pressure\ (parasites\ per\ unit\ time)}{Force\ of\ infection\ (insults\ per\ unit\ time)}$$

Complete analysis of the data was performed using the open source software in R [38].

Results

Exploratory analysis

The exploratory analysis of the data showed that foxes aged up to 3 years old, which represented 86% of the total samples, accounted for 88% of all infected animals and harboured 94% of the total parasite biomass. The study data encompassed 531 observations categorised by age of the host (juveniles, $n = 309$; adults between 1 and 2 years, $n = 99$; adults between 2 and 3 years, $n = 50$; and adults over 3 years, $n = 73$), type of urbanization zone (periurban, $n = 185$; border, $n = 200$; and urban, $n = 146$) and season when the fox was collected (spring, $n = 31$; summer, $n = 123$; autumn, $n = 113$; and winter, $n = 264$). The seasons were defined in groups of three months: spring (March to May), summer (June to August), autumn (September to November) and winter (December to February).

The parasite counts varied widely between observations with no parasites in 59% of the foxes, 21% foxes found with 1–99 worms, 17% foxes with 100–9,999 worms, and 3% of them with more than 10,000 worms. The proportion of parasite loads found in the foxes by type of urbanization zone, fox age and season are displayed in Table 2.

The distribution of *E. multilocularis* in foxes was highly aggregated (overall negative binomial constant $k = 0.05$).

Table 2 Observed proportions of *E. multilocularis* abundance (number of parasites/total number of parasites retrieved) in foxes in Zurich by type of urbanization zone, seasons and fox age

	Fox age (years)				
	< 1	1 to 2	2 to 3	> 3	Total
By Zone					
Periurban	0.51	0.02	0.01	0.04	0.58
Border	0.15	0.05	0.01	0.01	0.22
Urban	0.12	0.08	$2e^{-4}$	0	0.20
Total	0.78	0.15	0.02	0.05	1.00[a]
By Season					
Spring	$4e^{-3}$	$1e^{-5}$	0	0.01	0.01
Summer	0.08	$2e^{-5}$	$1e^{-5}$	$5e^{-5}$	0.08
Autumn	0.16	$1e^{-3}$	$4e^{-3}$	$2e^{-3}$	0.17
Winter	0.53	0.16	0.01	0.05	0.74
Total	0.78	0.16	0.01	0.06	1.00[a]

[a]Total number of parasites retrieved = 534,815 parasites

Model comparison

Transmission models comparing the possibility of acquired immunity or changes in infection pressure were compared to explore the hypotheses whether parasite induced immunity, seasonality, spatial differences and host age may be contributing to the observed pattern of parasite abundance in the foxes. A total of 20 models describing different scenarios for parasite transmission were compared based on their goodness-of-fit to the data and the number of parameters used, as it is illustrated in Fig. 2. The best fitting model was M20 (Table 3). Thus, models M1-M5 which described no spatial variation in transmission and models M6-M12 in which there were 2 spatial zones of transmission had a poorer fit to the data generally than models M13 to M20 where there were three spatial zones. Of these latter models those with a periodic infection pressure (M15-M20) described the data better than a non-periodic infection pressure (M13-M14). M20 and M19, with a decreasing abundance only in the periurban zone described the data better than M15 and M16 with either no decrease in infection pressure in any zone or a decrease in all 3 zones. M20 where the lower abundance in old foxes in the periurban zone is best explained by decreasing infection pressure in old fox gives a better description of the data than M19 where is hypothesizes it is due to parasite induced immunity. The difference between M17 and M20 is fixing the life expectancy of the parasite to that experimentally observed (M20) rather than using the data.

Best-fitting model

The best-fitting model, M20, assumed different parasite burdens in foxes from the periurban, border, and urban zones. The estimations of the negative binomial constants

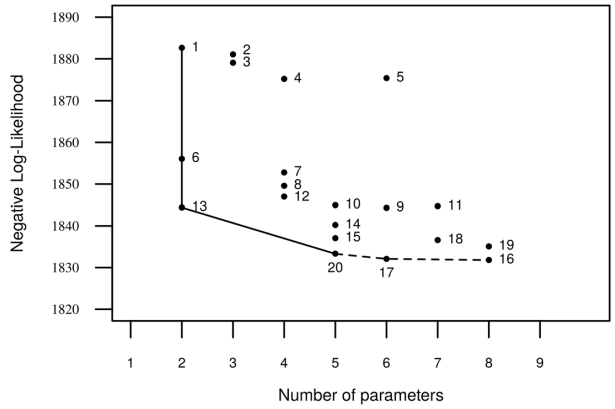

Fig. 2 Model comparison for *E. multilocularis* abundance models in foxes in Zurich. Model performance is assessed based on the smallest negative log-likelihood (NLL) for a given number of parameters used. Starting with the simplest model (Model 1), models along the lower left edge of the cloud (Models 1, 6, 13, 20, 17 and 16) were selected and compared pair-wise. Significant differences are shown as full line, while comparisons with results that did not have statistically significant differences are broken lines

indicated variability in the degree of aggregation of the parasites (k) between the three zones ($k_{peri} = 0.1$, $k_{border} = 0.02$, $k_{urban} = 0.05$). The model also considered age-dependent infection pressure, but only resulted in a better fit for such a model in the periurban area (i.e. old foxes had a lower exposure rate). In addition, the model suggested the existence of a sinusoidal infection pressure that varied with time with higher peaks during autumn and winter in foxes in all spatial zones, even though this seasonality was highest and most marked in the periurban zone. However, the baseline number of parasites (β_0) was found to be similar among the three zones and thus a single β_0 was applied to all zones. Finally, the model did not find evidence of parasite-induced immunity. Table 4 gives the maximum likelihood estimates (MLE) of the five parameters estimated by the model. Thus, the infection pressure, as described by equation (4), can be estimated at any time point (t) in any spatial zone. For example, a 10-month-old fox in the periurban zone has $\beta_0 = 8.5$, $\beta p = 2.6$ and $\phi = 0.5$ (t is in years, so in this case = 0.833). Thus $\ln[(h(t))] = 8.5 + 2.6*\sin(2*\pi*0.833)$ $-0.5*0.833 = 10.3$. Taking the exponent gives an infection pressure (or exposure) of 30,880 parasites per year at that time point. Likewise a 10-month-old fox in the urban zone has $\beta_0 = 8.5$ and $\beta u = 1.2$ ($\phi = 0$ as in model M20) and hence an infection pressure of 13,911 parasites per year.

A graphical representation of the seasonal variation of the infection pressure on each urbanization zone can be found in Fig. 3. The model gives predictions of the infection pressure greater than zero, even for newborn foxes, due to the baseline parameter (β_0). However, the fox cubs are not exposed to infection during their lactation period (*c.*4 weeks) thus foxes less than two months were assigned no parasites to their model predictions.

The most parsimonious model (M20) therefore indicated that there were spatial variations in infection pressure, with the periurban area having the highest value of h. The infection pressure varied throughout the year in all three spatial zones, with the highest infection pressure occurring in the winter months. Variations in abundance with age that were most notable in the periurban zone were better explained by an age-related decrease in infection pressure rather than prevention of reinfection by immunity resulting from an earlier exposure.

Parasites per infectious exposure

In periurban foxes, the maximum infection pressure occurred in winter and varied between 36,000 parasites in year 1 (1st winter), 22,000 in year 2 (2nd winter), and 13,000 in year 3 (3rd winter). This results in an approximate mean of 24,000 parasites per fox over the three winters. Lewis et al. [25], using the same data set reported around 9.5 infectious insults per year in winter. Therefore, about 2,500 parasites result from a single infectious insult in periurban foxes during winter. Likewise, in summer periurban foxes are exposed to an average of 230 parasites per year derived from 2.3 insults or 100 parasites per insult.

In urban foxes, we predict an infection pressure during winter of approximately 15,000 parasites per year from 1.8 insults or 8,300 parasites per infection event. In summer infections there are 1,500 parasites per year from 0.5 insults or approximately 3,000 parasites per infection event.

Table 3 Description and goodness-of-fit results of the abundance models for *E. multilocularis* in foxes in Zurich

Model	Description	P	NLL
One zone			
M1	Constant infection pressure	2	1,882.6
M2	Decrease in infection pressure and fox age	3	1,881.1
M3	Periodic relationship between infection pressure and fox age	3	1,879.1
M4	Periodic relationship between infection pressure and fox age plus a decreasing infection pressure with increasing fox age	4	1,875.3
M5	Periodic relationship between infection pressure and fox age plus a decreasing infection pressure with increasing fox age and also accounting for parasite-induced immunity	6	1,875.4
Two zones: periurban and suburban (border + urban)			
M6	Constant infection pressure	2	1,856.1
M7	Decrease in infection pressure and fox age	4	1,852.8
M8	Periodic relationship between infection pressure and fox age	4	1,849.6
M9	Periodic relationship between infection pressure and fox age plus a decreasing infection pressure with increasing fox age in both zones	6	1,844.3
M10	Periodic relationship between infection pressure and fox age plus a decreasing infection pressure with increasing fox age only in periurban zone	5	1,845.0
M11	Periodic relationship between infection pressure in both zones and only in the periurban area decreasing infection pressure with increasing fox age and parasite-induced immunity	7	1,844.7
M12	As M10, but μ^a was fixed as 8.6	4	1,847.0
	Three zones: periurban, border and urban		
M13	Constant infection pressure	2	1,844.4
M14	Decrease in infection pressure and fox age	5	1,840.2
M15	Periodic relationship between infection pressure and fox age	5	1,837.1
M16	Periodic relationship between infection pressure and fox age plus a decreasing infection pressure with increasing fox age in all three zones	8	1,831.8
M17	Periodic relationship between infection pressure and fox age plus a decreasing infection pressure with increasing fox age only in periurban zone	6	1,832.1
M18	Periodic relationship between infection pressure and fox age plus parasite-induced immunity in all three zones	7	1,836.6
M19	Periodic relationship between infection pressure and fox age in all zones and only in the periurban area decreasing infection pressure with increasing fox age and parasite-induced immunity	8	1,835.1
M20	As M17, but μ^a was fixed as 8.6	5	1,833.4

[a]Parasite death rate (μ)

Abbreviations: *P* model parameters, *NLL* negative log-likelihood values

Table 4 Maximum likelihood estimates (MLE) with a negative log-likelihood value of 1,833.4 of the abundance model parameters for *E. multilocularis* in foxes in Zurich with their 95% bootstrap confidence intervals (CI) for Model 20, with μ fixed at 8.6

Parameter	MLE	95% CI	
β_0	8.5	7.5–9.3	Baseline number of parasites of the infection pressure
β_p	2.6	1.4–4.1	Amplitude of the infection pressure in the periurban zone
β_b	0.1	-0.7–1.5	Amplitude of the infection pressure in the border zone
β_u	1.2	-1.3–2.7	Amplitude of the infection pressure in the urban zone
φ_p	0.5	0.3–1.3	Decrease parasite rate in the periurban zone

Abbreviations: *MLE* maximum likelihood estimates, *CI* confidence interval

Discussion

Model 20 was the best fitting model, so the data gives support to the hypotheses that there are: (i) spatial differences in parasite infection pressure among the three zones; (ii) temporal differences in parasite infection pressure in relation to time of the year and; (iii) there are infection pressure variations across different age groups only in the periurban area. These findings are consistent with some of the often interrelated and frequently reported risk factors in EM infection in foxes [40]. Nevertheless, some of the model implications are not in line with previous research. These findings are discussed further below in detail.

First, the model describes spatial differences in infection pressure across urbanization zones. Urban resident foxes in Zurich have been found to display small home ranges (*c.*25 ha) and they pursuit their daily activities mainly within this area, although some movement of foxes among urbanization zones also occurs [41, 42]. The level of urbanization of their limited territories determines the number of rodents and foxes and their predator-prey interactions, influencing ultimately parasite transmission [40]. Therefore, the model hypothesis of an existing heterogeneous distribution of infected foxes within the city is consistent with numerous studies that found an association between infection status and type of urbanization zone [40]. Even though this association has been often linked to other risk factors such as season [11, 16] and fox age [17]. Most of these studies also reported a gradual decrease in parasite prevalence from the rural areas and the periphery of the cities towards the more urbanised zones [27–29]. Similarly, Lewis et al. [25] estimated a higher number of infection exposures in periurban foxes (maximum of 9.35 to 9.7 insults/year) compared to urban foxes (maximum of 1.6 to 2 insults/year) in Zurich. Foxes from the outside and transition areas of the cities seemed to prey more on rodents and hence, be more exposed to parasite infection [17, 29]. This comes as a result of the presence of high densities of suitable intermediate hosts bearing high parasite prevalences in the outskirt of the cities [16, 17].

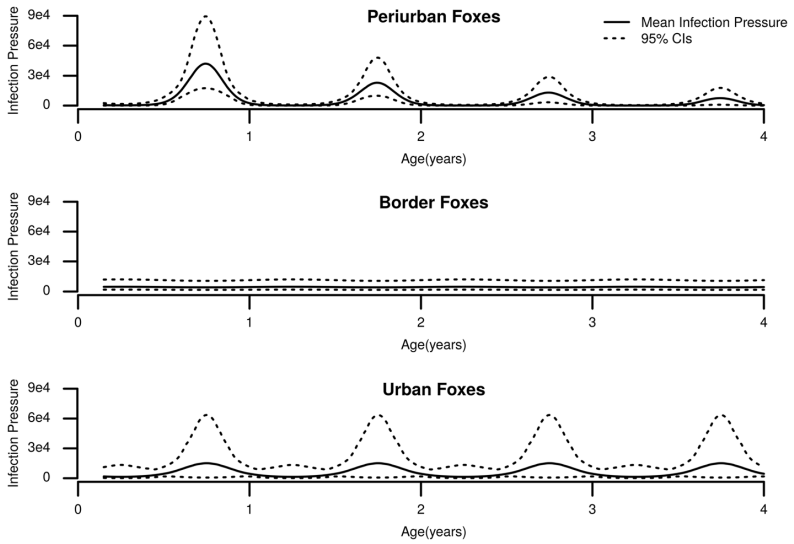

Fig. 3 Dynamics of *E. multilocularis* infection pressure (mean and 95% CIs) by fox age in the periurban, border and urban zones predicted by Model 20. The three plots show the variation in infection pressure in foxes by host age up to 4 years old. When fox age is used as a proxy of time the curve peaks correspond to the colder seasons (autumn and winter) separated by flat intervals, which correspond to the warmer seasons (spring and summer)

In contrast, urban foxes rely more on anthropogenic food for their diet, being highly abundant and accessible in the city center [14]. Similarly, our model estimated the highest amplitude to be in the periurban zone, implying that the highest infection pressure was borne by the periurban foxes. However, in our study this is just applicable to juvenile foxes since M20 describes the infection pressure in the periurban area is age-dependent resulting in adult foxes being exposed to less number of parasites per infection insult than their young. Consequently, only the periurban juveniles presented the highest infection pressure across all areas. In fact, the model predictions referring to adult foxes suggested the urban foxes harboured the highest infection pressure among zones. This is a surprising finding since it would be expected that animals living in the edge of the cities would prey more on rodents and thus be more exposed to infection as previously discussed. The model suggests that periurban adults are being infected more frequently on average, but that each infection event results in fewer parasites than a typical infection event in urban foxes. In absence of host immune responses to infection it may indicate that infected rodents that are consumed by urban foxes have greater numbers of protoscolices than those consumed by periurban foxes, even though urban foxes are consuming fewer infected rodents. This hypothesis differs to what has been reported in terms of parasite infection in city foxes [40]. A potential explanation might be that some super infected foxes collected in the urban area were in fact dispersal foxes whose home range encompassed mainly the border area but they died in the urban area during an excursion looking for feeding or mating opportunities. The occurrence of so-called floating individuals with larger home ranges has been previously recorded in Zurich [41]. These foxes are commonly young males that expand their range during the mating season (autumn and winter) [43]. In this case, seasonal variations in the spatial behaviour of foxes could explain the higher amplitude in the infection pressure found in the urban area. Alternatively, it could be due to the short history of urban colonization of foxes, suggesting that the transmission cycle is not yet equilibrated, showing typical flickering in transiting complex systems [44]. In any case, there is an increasing individual risk of developing AE mainly in areas where high densities of humans and urban foxes intersect [15, 24], which it is not the case of the city centre. The existence of a high infection pressure in the periphery of the cities and in the transition areas and adjacent spatial zones are still the main cause of concern in terms of AE transmission risk.

Secondly, the model also accounts for a sinusoidal infection pressure throughout the year with peaks during the cold months of autumn and winter. In Zurich, higher

infection rates have been previously recorded during winter, in association with the host age and the city zone where the fox was retrieved [11, 16]. Likewise, Lewis et al. [25] found a periodic force of infection with an annual minimum of 0.27–1.27 parasite insults and a maximum of 6.87–7.05 parasite insults per year in foxes collected in Zurich. Evidence of seasonal variation in prevalence in foxes has been frequently reported in other locations [26, 45, 46]. In fact, seasonal fluctuations in temperature and precipitation are often proposed as infection determinants because of their influence on hosts' numbers and parasite survival in the environment [40]. The low temperatures and humidity favour the survival of *E. multilocularis* eggs in the environment, potentially contributing to the occurrence of higher infection rates during the cold and rainy seasons [47]. In addition, the influence of climatic changes on the abundance and age-structure of vole populations [48–50] may have an impact on fox predation on voles [51] and consequently in the degree of parasite infection in foxes. Some studies have found that foxes showed higher predation on voles in autumn compared to spring coinciding with prey availability [11, 29]. Whereas, it has been reported a correlation between low day temperatures and higher infection rates in rodents [49, 50].

Thirdly, the model suggests the existence of an age-dependent infection pressure was found only in the periurban area. Periurban foxes prey more on rodents compare to other urbanised zones [17], thus if there is any effect of fox age in parasite exposure it is more likely to be evident in this area. In Zurich, higher worm burdens have been recorded in younger foxes compared to adults [11]. In addition, seasonal variations in prevalence were more marked in the juvenile animals in the same Swiss city [17]. Despite being a young fox has been repeatedly reported as an infection determinant for *E. multilocularis* [40], the underlying cause remains unclear. Potential reasons for decreasing parasite abundance in the periurban adult fox may include predatory behaviour or diet preferences. Juveniles might have a higher proportion of rodents in their diet, as they are abundant and easy to prey whereas adult foxes might have better access to more difficult prey or have more experience finding food from anthropogenic sources. Alternatively, inexperienced juveniles might be inclined to prey on infected voles if parasite infection adversely affects the intermediate host [52]. Nevertheless, although variation in feeding behaviour across age groups of foxes has not been demonstrated, it has been hypothesized that juvenile foxes may have best access to voles [17]. The dietary response of red foxes is complex when abundant alternative resources are available such as anthropogenic food and multiple intermediate host species [53]. The diet of urban foxes has a dominance of scavenged meat and other scavenged and cultivated fruit and crops with more than half of an average stomach content being anthropogenic. The proportion of scavenged food recovered from foxes' stomachs increases in foxes found in the city center compared to the periurban area [14, 17]. Such a variation in fox dietary preference correlates with the spatial variations in infection pressure reported in the best fitting model M20.

Other proposed explanation for age-related differences in burdens of parasites considers the existence of a developing immunological response after repeated infection [11, 26, 54]. There are previous studies in highly endemic regions for *E. granulosus* which document a negative correlation of parasite abundance with age in dogs which would be predicted if parasite-induced host immunity limited infection [31, 55, 56]. In addition, experimental infections have shown evidence of parasite-specific antibody responses in animal hosts although it remains unclear their effect on parasite infection [57]. Nonetheless, previous models assuming presence of acquired host immunity did not give a better fit to *E. multilocularis* data in dogs or foxes [25, 55]. Furthermore, studies in Kyrgyzstan and Lithuania failed to demonstrate a decrease in *E. multilocularis* abundance with increasing fox age [58, 59]. Moreover, in our study the parameter values on which the models incorporating immunity converged indicated a very high value of γ – the rate of loss of immunity. This would indicate that the duration of immunity following exposure would only be a matter of weeks at most, and require conditions of extremely high infection pressure to be maintained. Even if immunity were present its effect on parasite abundance would be negligible with this SIR model. Thus, the better fit to data given by the models without immunity or the potential very high rates of loss of immunity, if present, are evidence that definitive host immunity is not regulating the parasite population in this system.

Experimental studies where foxes were artificially infected have reported a pre-patent period of 29–33 days [60] and a patent period of up to three months [61]. In the study of Kapel et al. [36] it took approximately 42 days for foxes experimentally infected with 20,000 protoscolices to reduce their worm load to 50%. We used this measure to calculate the parasite death rate in models M12 and M20 as the data itself was not able to define this parameter well (Table 3).

We have attempted to quantify the infection pressure of *E. multilocularis* in foxes in Zurich to gain a better insight on parasite epidemiology through hypothesis testing using a relatively simple transmission model. The modelling of the *E. multilocularis* infection is potentially a complex task since the dynamics of parasite transmission are influenced by a wide range of interrelated factors such as, hosts' population densities, predator-prey

interactions, landscape characteristics, climate conditions and human-related activities [40]. Additionally, the modelling of parasite abundance brings extra challenges due to the extreme aggregation of parasites within their hosts. This intense aggregation produces a high degree of uncertainty to the model predictions. This is reflected in the wide confidence intervals related to the model predictions. Such wide confidence intervals could have been narrowed if the data set had had more data points. However, despite this the major findings of the study are robust as the most parsimonious model had an improved likelihood (or statistical fit) compared to the competing models (representing competing hypotheses). Furthermore, the basic model was first proposed by Roberts et al. [30] and has since been used on several data sets [31, 56, 62] and has proved robust, despite its relative simplicity. The present study introduced potential seasonal variations in infection pressure by allowing the parameter h to have a sinusoidal relationship with age (and hence time). Other data sets analyzed with this model were taken at 1 time point and thus could not analysed in such a way. Thus, in terms of the hypotheses we tested the model appears to have validity and robustness, although it would further support our findings if or when another similar data set becomes available to confirm this.

Nevertheless, models are conceived to be a simplified representation of the highly complex processes in nature and provide a useful tool to assess different hypotheses. All models are wrong, but the question remains as to how wrong the model must be to lose its usefulness [63]. Given the assumptions in the model, the data suggests spatial and age-related variations in infection pressure to foxes. Therefore, even considering all limitations, the model offers a practical platform to improve knowledge on parasite epidemiology and to allow the quantification of epidemiological parameters that cannot be measure directly in the field, such as the infection pressure. Some of the implications derived from the model concurred with previous epidemiological knowledge on *E. multilocularis* infection, such as the existence of spatial heterogeneities, seasonal fluctuations and age-related differences. Alternatively, other conclusions diverged from previous reports, such as finding that the highest number of parasites developing in the fox after infection exposure occurs in the urban area. However, it cannot be ruled out the possibility that the few urban foxes found harbouring high loads of parasites might have become infected in the neighboring area previously to their incursion into the urban zone. The model also challenged the hypothesis that parasite-induced host immunity may play a role in the transmission dynamics of *E. multilocularis*. Using the models described we found no convincing evidence that this may be the case. The decrease in abundance in foxes, only observed in the periurban zone, is better explained by a decrease in infection pressure in older foxes, although the differences in model predictions are quite subtle.

Conclusions

In conclusion, the model gives a picture of the overdispersed infection pressure borne by foxes in Zurich, highlighting the potentially large contribution of young periurban foxes and foxes from the outside perimeter of urban areas towards environmental contamination. Previous studies have proved the efficacy of the use of anthelmintic baiting to reduce the environmental contamination with parasitic eggs [64–66]. Similarly, in Zurich the placement of monthly baits along the urban periphery has been shown to successfully decrease the amount of coproantigen-positive fox faeces and reduce infection rates in intermediate hosts (*A. terrestris*) in baits areas [67]. However, temporal anthelmintic interventions mostly failed to achieve permanent parasite elimination [64, 68]. Hence, there is a need to ensure the optimisation of potential long-term baiting campaigns [19]. Model results suggest that a reduction in parasite biomass in Zurich foxes could be more effectively achieved if baiting strategies were to be intensified in the periphery of the city and during the autumn and winter months. The quantification of the temporal-spatial variation of the number of parasites in foxes can help to optimise the designing of targeted bait programmes aiming to reduce the level of environmental contamination and ultimately, infection risk in humans.

Additional files

Additional file 1: Data S1. File containing original data. (XLSX 20 kb)
Additional file 2: Text S2. R code. (DOC 44 kb)

Abbreviations

AE: Alveolar echinococcosis; CI: confidence intervals; MLE: maximum likelihood estimate; NLL: negative log-likelihood

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Availability of data and materials

The datasets are available within the article and its Additional files.

Authors' contributions

BOA and PRT conceived and designed the study. DH and PD undertook associated studies which supplied the data. BOA, SRR and PRT undertook

the analysis. All authors contributed to drafting of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Not applicable.

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Paper 4

Latent class models for *Echinococcus multilocularis* diagnosis in foxes in Switzerland in the absence of a gold standard

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Abstract

Background

In Europe the principal definitive host for *Echinococcus multilocularis*, causing alveolar echinococcosis in humans, is the red fox (*Vulpes vulpes*). Obtaining reliable estimates for the prevalence of *E. multilocularis* and relevant risk factors for infection in foxes can be difficult if diagnostic tests with unknown test accuracies are used. Latent-class analysis can be used to obtain estimates of diagnostic test sensitivities and specificities in the absence of a perfect gold standard. Samples from 300 foxes in Switzerland were assessed by four different diagnostic tests including necropsy followed by sedimentation and counting technique (SCT), an egg-PCR, a monoclonal and a polyclonal copro-antigen ELISA. Information on sex, age and presence of other cestode species was assessed as potential covariates in the Bayesian latent class models. Different Bayesian latent-class models were run, considering dichotomized test results and, additionally, continuous readings resulting in empirical ROC curves.

Results

The model without covariates estimated a true parasite prevalence of 59.5% (95%CI, 43.1–66.4). SCT, assuming a specificity of 100%, performed best among the four tests with a sensitivity of 88.5% (95%CI, 82.7–93.4). The egg-PCR showed a specificity of 93.4% (95%CI, 87.3–99.1), although its sensitivity of 54.8% was found moderately low (95%CI, 48.5–61.0). Relatively higher sensitivity (63.2%, 95%CI 55.3–70.8) and specificity (70.0%, 95%CI 60.1–79.4) were estimated for the monoclonal ELISA compared to the polyclonal ELISA with a sensitivity and specificity of 56.0% (95%CI, 48.0–63.9) and 65.9% (95%CI, 55.8–75.6), respectively. In the Bayesian models, adult foxes were found to be less likely infected

than juveniles. Foxes with a concomitant cestode infection had double the odds of an *E. multilocularis* infection. ROC curves following a Bayesian approach enabled the empirical determination of the best cut-off point. While varying the cut-offs of both ELISAs, sensitivity and specificity of the egg-PCR and SCT remained constant in the Bayesian latent class models.

Conclusions

Adoption of a Bayesian latent class approach helps to overcome the absence of a perfectly accurate diagnostic test and gives a more reliable indication on the test performance and the impact of covariates on the prevalence adjusted for diagnostic uncertainty.

Keywords:

Echinococcus multilocularis, foxes, diagnostic test, diagnostic sensitivity, diagnostic specificity

INTRODUCTION

Echinococcus multilocularis is a zoonotic tapeworm found in the northern hemisphere and mainly transmitted between foxes and small mammals [1]. Humans are accidental hosts that can become infected through the oral intake of parasite eggs. In the absence of treatment, potentially fatal alveolar echinococcosis (AE) develops [2]. There is evidence of a geographic expansion of the known *E. multilocularis* endemic area in Central Europe towards the north, west and east of the continent [1]. Expert consensus

foresees a delayed increase in the occurrence of AE cases in Europe within the next decades due to its long incubation period [3]. In consequence, information on the parasite distribution in the red fox (*Vulpes vulpes*), the principal definitive host in Europe of *E. multilocularis*, is paramount to estimate the potential risk of human infection and assist in prevention efforts [4, 5]. Three of the diagnostic techniques frequently used for *E. multilocularis* detection in the definitive host include: the visual identification of adult worms in the small intestine at necropsy through the sedimentation and counting technique (SCT), the parasite coproantigen detection and the amplification of DNA from parasitic eggs present in the fox faeces [6]. The performance of these tests, for a given population, are commonly measured based on their diagnostic sensitivity and specificity. The necropsy followed by SCT is considered the reference test with a very high specificity (around 99%), as the morphological features of *E. multilocularis* allow an unequivocal diagnosis in most cases [7]. However, some limitations concerning SCT's sensitivity must be taken into consideration [8, 9], as high worm burdens are required. Despite some available modifications in its performance [10, 11], this technique remains laboratory intensive, time consuming and expensive, and entails the implementation of strict safety precautions to minimize the risk of infection of the personnel involved. In addition, this procedure requires the collection of dead red foxes limiting its practicality for population studies. The detection of parasite antigens in the fox faeces through the binding of antigen-antibody in an enzyme-linked immunosorbent assay (ELISA) remains an alternative method for the diagnosis of parasite infection in foxes. The coproantigen test has the advantage of detecting also pre-patent infections [12–14]. Polyclonal- and monoclonal-antibody-based ELISAs have been developed for the detection of *E. multilocularis* [12, 13, 15, 16]. High sensitivities (≈ between 80–95%)

and specificities (≈ between 70–99%) have been originally reported for the coproantigen test [12, 13] although sensitivities are strongly dependent on fox worm burdens [13, 17–19]. Being a relatively safe, rapid and inexpensive test it qualifies as a potential technique for mass screening in the fox population from endemic areas where false positives are acceptable. The parasite distribution is known to be skewed with a small number of foxes harbouring a high number of worms [20]. It is believed that foxes with moderate to high worm burdens might contribute to most of the environmental contamination and hence, to human exposure [21]. Thus, it is paramount that the diagnostic test could adequately identify them. Consequently, the present study included a scenario where foxes were harbouring worm loads of 100 or more parasites to evaluate the potential performance of one of the coproantigen test for population studies. A third diagnosis option is the detection of *E. multilocularis* genetic material excreted with the faeces of the definitive host through the amplification by the polymerase chain reaction (PCR). Since the first publication of this technique for *E. multilocularis* diagnosis [22] different approaches have been developed to improve its performance on faeces [23–31]. This method is highly specific, but low worm burdens and the presence of inhibitory components may compromise its sensitivity [29, 32]. However, these limitations might be overcome after the development of newly magnetic capture-PCR and the implementation of real-time PCR procedures assigning this diagnostic procedure with a sensitivity comparable to SCT's [9]. Nevertheless, it remains a labor intensive and expensive technique so its application in population studies is commonly restricted as a confirmatory test for coproantigen positive samples [13, 14, 26]. Despite several available *E. multilocularis* diagnosis options in foxes none of them can be regarded as a perfect gold standard test, with 100% specificity and 100% sensitivity. Therefore,

prevalence studies in foxes rely on imperfect diagnostic methods and these limitations in tests' accuracies should be taken into account when reporting and interpreting their results [6].

A widely used approach to overcome the lack of a perfect gold standard test is through the application of latent class models, using frequentist or Bayesian methods. Hui and Walter [33] originally described the latent class models using a frequentist approach by first considering the case where two tests were applied to two populations with different prevalences, under the assumption of sensitivities and specificities being constant across populations and conditional independence between the two tests. Hui and Walter also showed that, given the model assumptions are met, if the condition of $S \geq R / (2^{R-1} - 1)$ is satisfied, where S represents the number of populations and R the number of tests applied, there will be enough degrees of freedom to estimate the parameters of interest. Since then, derivations of the Hui and Walter model have been developed to estimate the unknown parameters that are *latent* in the data when a gold standard test is not available [34]. When Bayesian approaches are implemented prior information can be incorporated and potentially conditional dependencies assessed. The evaluation of the accuracy of the diagnostic methods for *E. multilocularis* detection by latent class analysis has become increasingly common [9, 31, 35].

In the present study we applied Bayesian Latent Class Models using the results of four diagnostic tests for *E. multilocularis* in foxes, the necropsy and SCT, the monoclonal ELISA, the polyclonal ELISA and the egg-PCR, to a single reference fox population in Switzerland aiming to address the following research questions: i) what is the true parasite prevalence?; ii) what are the performance characteristics of the diagnostic tests?; iii) have any of the three covariates assessed (fox age, sex and presence of co-

infection with other cestodes) an effect on the true infection status?; iv) do any differences exist between the selection of the cut-off point for the ELISA by adopting Bayesian latent class models compared with the employment of the classic method of considering the necropsy and SCT as the gold standard test?; v) has the selection of the ELISA cut-off point any effect on the estimation of performance of the other tests?; and vi) what is the impact on the performance of the monoclonal-ELISA if we change the threshold for the necropsy and SCT results to be considered a sample positive only with 100 or more *E. multilocularis*?

MATERIALS AND METHODS

Fox samples

A total of 300 red foxes (*Vulpes vulpes*) were examined at the Parasitology Institute, University of Zurich, for *E. multilocularis* as part of the European Research Programme on Emerging and Major Infectious Diseases of Livestock (EU-Project EMIDA). The animals were shot and collected by hunters at different locations in the midlands of Switzerland during the official hunting seasons between 2012 and 2014. Thus, it is representative of this area and not of the alpine regions, which tend to have a lower prevalence of infection. According to the Swiss Animal Welfare act, article 3, this research project is not considered as an animal experiment. Due to the risk associated with the handling of infectious materials, a fraction of the small intestines retrieved from the fox carcasses were frozen at -80°C for five days before proceeding with their parasitological examination [36]. However, 163 of them were only kept at 4°C as there was a need to collect viable *E. multilocularis* eggs for experimental

infection of rodents in the context of the EMIRO project, a research project in the framework of the EMIDA ERA-NET [37].

Diagnostic tests

Four diagnostic procedures were performed for each fox. The original data file with the diagnostic test results and information of covariates can be found in the additional file 1.

Necropsy and Sedimentation counting technique (SCT)

The small intestines were removed during the necropsy of the fox carcasses to later be used for the identification of adult stages of *E. multilocularis* by SCT. This procedure was carried out as previously described in [20]. The suggested sensitivity of this procedure is 98% [38]. Results were recorded for fox classification as positive (1) or negative (0) for *E. multilocularis* presence. During necropsy, information related to sex of the fox, presence of other cestode species and fox age was recorded for each animal. This information was registered by assigning numerical values of 0 and 1 as follows: female = 0 and male = 1, young = 0 and adult = 1 and absence of cestodes = 0 and presence of cestodes = 1. The proportion of foxes by age, sex and presence of cestodes co-infection are displayed in Table 1. The age determination of the fox was roughly estimated based on the displaying level of tooth wear [39]. Animals with front upper incisors showing a sharp and visible fleur-de-lys pattern were regarded as young foxes (< 1 year old) while animals displaying a high degree of attrition were classified as adults (> 1 year old). In addition, fresh faecal samples were collected from the rectum of each fox and kept at -80°C for at least one week before being processed.

196

197 *Coproantigen enzyme-linked immunosorbent assay (ELISA)*

198 Part of the faecal samples was analysed using two coproantigen tests, specific for *E.*
199 *multilocularis* diagnosis. Both ELISAs have been produced by the Institute of
200 Parasitology of Zurich: the polyclonal antibodies based ELISA (pAb- ELISA) using
201 rabbit and chicken egg antibodies was performed as described [13] and the recently
202 modified monoclonal antibody based ELISA (mAb-ELISA) using a rat monoclonal
203 antibody directed against *E. multilocularis* integument antigen and rabbit antibodies
204 as described [40]. The ELISAs results were expressed in corrected A405nm reading
205 values obtained from the subtraction of the specific reaction minus the unspecific
206 reaction [40]. The original overall reported sensitivity of the pAb-ELISA, calculated
207 as the mean A_{405nm} reading value plus 3 times the standard deviation of fecal samples
208 or intestinal contents of *Echinococcus*-free dogs and foxes, was 84%, strongly
209 dependent on worm burdens [13]. The ELISAs results were classified as positive (1)
210 or negative (0) considering the necropsy and subsequent SCT as the perfect gold
211 standard test. The Receiver Operating Characteristic (ROC) curve was built by
212 comparing the ELISA's numerical continuous reading values to the dichotomous
213 necropsy and SCT results by using the pROC R package [41].

214

215 *Copro-DNA detection by multiplex polymerase chain reaction*
216 *(egg-PCR)*

217 The remainder of the faecal material was used for the isolation and microscopy
218 identification of taeniid eggs as described in [24], followed by egg-DNA extraction
219 and egg-DNA detection by a multiplex PCR following indications of [27].

The original proposed sensitivity for this procedure, estimated by comparison with the results derived from the microscopic examination of the deep intestinal mucosal scrapings after necropsy, was 89% dependent on worm burdens and the maturity of the worms [25]. The combination of egg isolation and egg-DNA detection by PCR gave the information to classify the samples as positive (1) or negative (0) for *E. multilocularis* infection.

Bayesian latent class models

The test results on *E. multilocularis* infection in foxes were analysed using latent class models within the Bayesian framework described in detail in [42]. The aim of this approach is to identify appropriate models, which jointly estimate the diagnostic test accuracies, conditional dependencies and disease prevalence and simultaneously to identify those covariates which are related to the true prevalence (and not solely to the apparent prevalence) in the absence of a true gold standard. The probability model used is the binomial distribution to model prevalence. The description of the model code used for the analysis of three and four diagnostic tests is available in the additional files 2 and 3.

Latent class analysis for three tests

The first part of the latent class analysis included the results of three of the diagnostic tests: necropsy and SCT, pAb-ELISA and egg-PCR. The model parameters encompassed: the true parasite prevalence, the sensitivities and specificities of the three diagnostic tests (Se_1, Se_2, Se_3, Sp_1, Sp_2, Sp_3) and their corresponding two-way covariance terms. With the aim to adjust for conditional dependencies, first all potential covariances (σSe_{12}, σSe_{23}, σSe_{13} and σSp_{23}) were included simultaneously.

Subsequently, in the absence of evident covariances (i.e. the posterior mean was equal to zero), they were set to 0. Since the specificity of the necropsy and SCT has been reported to be close to 99% [36] this parameter (Sp_1) was fixed to 1.

Latent class analysis for four tests

The second part of the latent class analysis included the results of the four diagnostic tests: necropsy and SCT, pAb-ELISA, mAb-ELISA and egg-PCR. The model parameters encompassed: the true parasite prevalence, the sensitivities and specificities of the four diagnostic tests (Se_1, Se_2, Se_3, Se_4, Sp_1, Sp_2, Sp_3, Sp_4) and their covariance terms. Once more the specificity of the necropsy and SCT was fixed to 1. Similarly, first all potential nine covariance terms (σSe_{12}, σSe_{23}, σSe_{34}, σSe_{13}, σSe_{14}, σSe_{24}, σSp_{23}, σSp_{24} and σSp_{34}) were included simultaneously, and set to 0 subsequently, when the posterior means were equal to zero.

Model priors

Non-informative beta priors (1,1) as well as informative beta priors were selected for the *latent* prevalence and the test sensitivities and specificities, as beta distributions are well suited to describe the uncertainty associated to a binomial probability. The software Betabuster was used to obtain the values for the informative priors based on literature. The informative priors are presented in the additional files 4 and 5. A sensitivity analysis was performed to assess a potential influence of the priors on the posteriors and assess the robustness of the results. The sensitivity analysis consisted of varying the informative priors for each of the parameters of interest, one at a time, while keeping the other priors fixed for both the three- and the four-test models. We varied the informative prior of the parameter of interest systematically from assuming

that the parameter is larger than 0.9, 0.8 and so on until 0.1, with a respective mode of 0.95, 0.85 and so on until 0.25. With this approach we obtained a number of informative priors, ranging from strong priors with a small variance (steep curve) or high precision, e.g. “greater than 60 % with a mode at 65%” to rather uninformative priors e.g. “greater than 10% and a mode at 95%” (flat curve). The latter one is close to the uninformative priors dbeta(1,1). Furthermore, with this approach we also obtained a number of priors, which are – potentially - in conflict with our data, e.g. we assume that the sensitivity is not close to 95% or 25%. Results of the sensitivity analysis for sensitivity of PCR in the three-test model are shown in the additional file 6. The covariance terms were assumed to be uniformly distributed ranging from -1 to 1.

Model fitting and comparison

Latent class models were fitted using Markov chain Monte Carlo (MCMC) simulation by employing the free statistical software JAGS version 3.1.0 [43]. For each model, three chains of the Gibbs sampler were run independently for 200,000 iterations after an initial burn-in of 50,000 iterations. The behavior of the MCMC chains was monitored through the plotting of the posterior values to identify potential converging problems. The output files from the Gibbs sampler were analyzed through the package *coda* [44] calculating the multivariate potential scale factor within the open source software *R* [45]. The model comparison of goodness-of-fit to the data was based on three criteria. The first criterion included the histograms resulting from the marginal posterior distribution for each covariance term. If the histograms showed the higher frequencies around 0, and the posterior mean was zero, then it was assumed that this term was negligible and thus, its addition did not improve the model. The

second criterion was based on the impact experimented by the parameters estimates and their credibility intervals following the addition of a covariance term. The parameter point estimates were reported as the mean of their marginal posterior distributions. If the parameter estimates did not vary greatly it indicated the redundancy of adding the extra term to the model. The third criterion was based on the deviance information criterion (DIC), which takes into account the deviance of the posterior mean of the parameters and the effective number of parameters used in the model. The smaller the value of the DIC, the better the model fits the data without over fitting.

Model with covariate pattern

The three covariates, *Sex*, *Age* and presence of other *Cestodes*, were included to the best model one at a time to explore their potential association with the fox infection status. We used a binomial regression model with a logit link function between the true unknown prevalence and the covariate term including an intercept and a slope. The improvement of the model after adding each covariate was established if there was a significant reduction in the DIC (by at least 2 units) and depending on the impact on the parameter estimates and accuracies. The covariates were regarded as statistically significant associated to *E. multilocularis* infection when the credibility intervals of the slope (expressed in odds ratio) did not include 1. The three MCMC chains ran independently for 200,000 iterations after a burn-in of 50,000 iterations and the plots of the posterior values for each chain were visually checked to identify potential converging problems and multivariate potential scale factors were obtained.

The Receiver Operating Characteristic (ROC) curve

The ROC curve describes graphically the ELISA performance by plotting the sensitivity on the y-axis against 1-specificity on the x-axis for many different cut-off points. The area under the ROC curve (AUC) provides an overall measure of the accuracy of the ELISA. We produced first two ROC curves: one for the pAb-ELISA and one for the mAb-ELISA with the model for three tests. Subsequently, two ROC curves for both ELISAs with the four-test model, including the cut-offs estimated from the previous analyses, were generated.

Bayesian empirical pAb- and mAb-ELISA ROC curves

The ROC curves for the ELISA tests were produced by considering the results of three tests in first place, to then consider the results of all four tests together. For the analyses including three of the tests two ROC curves were produced: one curve based on the results of the necropsy and SCT, pAb-ELISA and egg-PCR and the other curve based on the results of the necropsy and SCT, mAb-ELISA and egg-PCR. To that end, a hundred potential cut-off values were obtained from the percentile values of the ELISAs' optical readings (*Specific* minus *Unspecific*), ranging from the 1st to the 100th. For each of these 100 cut-off points, the results of the pAb- and mAb-ELISA were classified as positive or negative. Therefore, a hundred different classifications were obtained for the results of both ELISAs. Next, the best-fitting model (without covariates) was run 100 times using one of these hundred classifications obtained for the ELISAs' results each time. Afterwards, the estimated values of the sensitivities and specificities for both ELISAs obtained from the model were used to produce the two ROC curves for 100 possible cut-off points. Next, the same procedure was carried out to produce the ROC curves for the ELISAs but now the results of all four tests were included in the analysis. In addition, this time the value used to classify the

results of the ELISAs were the best cut-off determined in the previous three-test models

Bayesian empirical mAb-ELISA ROC curve after changing the threshold for the necropsy and SCT

Finally, we changed the threshold criteria for the necropsy and SCT results by assigning a positive value only to the fox samples where 100 or more parasites were counted. The best-fitting model (without covariates) to the results of the four diagnostic tests was run a hundred times, following the same procedure as above, to produce a new mAb-ELISA ROC curve.

RESULTS

Bayesian latent class models for three diagnostic tests

Since the posterior means of the three sensitivity covariance terms were clearly distinct from zero, they were included in the final model and are presented in the additional file 9. In contrast, due to the absence of evident covariance (posterior mean equal to zero), the specificity covariance between PCR and pAb-ELISA was set to 0. The addition of sensitivity covariance terms compared to the independence model, without any covariances included led to a decrease of approximately 2% points in the posterior means.

The estimated parameter values with their 95% credibility intervals and DIC for the best-fitting model with and without covariates are presented in Table 2. Figures 1 and 2 show estimated true *E. multilocularis* prevalence in foxes with and without the significant covariates, *Cestodes* and *Age*.

Two covariates, *Cestodes* and *Age*, were found significantly associated with *E. multilocularis* occurrence in foxes. The addition of the covariate *Cestodes* brought the largest improvement in DIC and suggested that foxes with a concomitant cestode infection had double the odds of presenting *E. multilocularis* compared to foxes without it. The model including the covariate *Age* experienced a less remarkable improvement in DIC and implied that adult foxes were less likely to be infected with *E. multilocularis* compared to younger animals. The covariate *Sex* was found not significant, with no differences in *E. multilocularis* infection between males and females. The addition of covariates to the model had a negligible influence on the parameter estimates.

Bayesian latent class models *for four diagnostic tests*

Similarly to the three-test models, all six sensitivity covariances had posterior means unequal to zero and were therefore included in the final model (additional file 9). In contrast, there was no evidence for covariances between specificities (i.e. posterior mean equal to zero), and all three potential specificity covariances were set equal to 0. The parameters estimates with their related 95% credibility intervals and DIC for the best-fitting model with and without covariates are presented in Table 3. Figure 3 and 4 show the *E. multilocularis* prevalence in foxes with and without the significant covariates as well as *Cestodes* and *Age*.

Once more, the covariates *Cestodes* and *Age* were found significantly associated to *E. multilocularis* presence in the fox. Again, the model including the covariate *Cestodes* displayed the lowest DIC indicating that the odds of *E. multilocularis* infection doubled in foxes with concurrent cestode infection in comparison to foxes without it. The covariate *Age* was also found significant although its addition to the model did

not cause a remarkable reduction in the DIC. The model suggested lower odds of *E. multilocularis* infection in adults than younger foxes. The covariate *Sex* was found not significant, with no differences in *E. multilocularis* infection between male and female foxes. The addition of covariates to the model did not change the parameter estimates.

The Receiver Operating Characteristic (ROC) curve results

Bayesian empirical pAb-ELISA ROC curve from the three-test model

The best cut-off point obtained from the pAb-ELISA ROC curve using the classical method of considering necropsy and SCT as a gold standard test was 0.21, assigning the coproantigen test with 58.5% sensitivity, 65.4% specificity and an overall accuracy of 63.8% (95%CI 57.6 to 70.1%) given by the AUC. The optimal cut-off value from the Bayesian pAb-ELISA ROC curve using the three-test model was 0.29, assigning the coproantigen test with 42.2% sensitivity, 77.8%, specificity and an overall accuracy of 60.7% given by the AUC. Figure 5 shows both pAb-ELISA ROC curves derived using the classical and the Bayesian approach.

Bayesian empirical mAb-ELISA ROC curve from the three-test model

The best cut-off point obtained from the mAb-ELISA ROC curve using the classical method was 0.10, assigning the coproantigen test with 65.2% sensitivity, 68.4% specificity and an overall accuracy of 71.2% (95%CI 65.4 to 77.0%) given by the AUC. The optimal cut-off value from the Bayesian mAb-ELISA ROC curve using the three-test model was 0.16, assigning the coproantigen test with 68.3% sensitivity,

75.3% specificity and an overall accuracy of 71.7% given by the AUC. Figure 6 shows both mAb-ELISA ROC curves derived using the classical and the Bayesian approach.

Bayesian empirical pAb- and mAb-ELISA ROC curves from the four-test model

When including the mAb-ELISA cut-off based on a Bayesian approach in the four-test model, the AUC for the pAb-ELISA ROC curve was similarly to the three-test model, e.g. 60.7%. The highest sum of the sensitivity plus specificity, was 1.20 with an associated sensitivity and specificity of 69.9% and 50.6%. The corresponding cut-off was 0.17. The second highest sum of sensitivity and specificity was 1.19 with the same cut-off as in the three-test model of 0.29. For this cut-off the sensitivity and specificity were 41.6% and 78.3%.

When including the pAb-ELISA cut-off based on a Bayesian approach in the four-test model, the AUC for the mAb-ELISA ROC curve was 76.2% for the same cut-off 0.16 with associated sensitivity and specificity of 70.5% and 80.0%. In the additional files 7 and 8 the ROC curves for both ELISAs with the classical and the Bayesian approach are shown.

The variation of the cut-off points for the classification of both ELISA tests, pAb- and mAb-ELISAs had virtually no impact on the estimations of the other parameters of interest. The analysis was performed once more using the four-test model and a new classification for the necropsy and SCT results, being positive only the samples with 100 or more *E. multilocularis*. In this case the optimal cut-off point determined by the Bayesian mAb-ELISA ROC was still 0.16, conferring to the coproantigen test with

70.5% sensitivity, 80.0% specificity and an overall accuracy of 76.2% given by the AUC. Figure 7 shows the corresponding mAb-ELISA ROC curve.

DISCUSSION

The employment of latent class models to analyse the results of the diagnostic tests for *E. multilocularis* allowed the determination of the tests performance in the study population and the estimation of the true parasite prevalence in the absence of a perfect gold standard test. Furthermore it was also possible to adjust for potential conditional dependence between tests. In addition, these models could evaluate the association between three covariates and parasite infection occurrence in the fox. Likewise, the application of latent class models permitted the building of the ROC curves for the ELISAs following a Bayesian approach that enabled the empirical determination of the best cut-off point and the evaluation of the impact that the selection of the cut-off had on the estimation of the rest of the tests characteristics.

In the present study, the latent class models including all potential covariances between sensitivities proved to be robust and their parameter estimates showed to be consistent with previous knowledge. The point estimates for the true *E. multilocularis* prevalence in foxes given by the three and four-test models (without covariates) were 58.4% and 59.5%, respectively. Similar high parasite prevalences have been previously reported in Swiss foxes [46–48]. In regard to the tests performances, the model estimates are also in line with prior information on diagnostics accuracy of these techniques. The best-fitted models (without covariates) gave high point estimates for the necropsy and SCT sensitivities, 91.9% and 88.5%. The SCT's sensitivity has commonly been considered relatively high, 98 to 100% [38] since the

469 immersion of the intestines in saline solution and the posterior scrapping of the
470 intestinal wall ensures the release of most of the worms [36]. Hence, if a fox has
471 intestinal worms this method should identify them reliably. However, an experimental
472 study determined that intestinal samples should contain at least 10 tapeworms to
473 achieve a 60% probability of obtaining positive detection [8]. Although experimental
474 conditions differ from natural infection, this study highlights SCT's sensitivity
475 limitations related to worm burdens. In addition, the combination of worms'
476 degradation during post-mortem conditions plus the intestines deep-freezing stage
477 involved in the SCT process could also affect the SCT's sensitivity. Moreover, a
478 recent latent class analysis of *E. multilocularis* diagnostic tests estimated the SCT-
479 sensitivity to be between 76 to 88% [9]. Hence, SCT should not be regarded as a
480 "gold standard" test [6]. The estimated specificities of the pAb-ELISA from the three
481 and four-test models (without covariates) ranged between 54.0 to 73.2% and 55.8 to
482 75.6%, respectively. The estimated specificity of the mAb-ELISA was found amid
483 60.1 to 79.4%. Coproantigen specificities can be altered by the occurrence of cross-
484 reactions with antigens from concomitant helminths infections [13] or even the
485 persistence of *E. multilocularis* antigens in the faeces after the fox is no longer
486 infected resulting in false positives results. The pAb-ELISA and the mAb-ELISA's
487 estimated sensitivities from the three and four-test models (without covariates) ranged
488 between 47.5 to 63.7% and 48 to 63.9% for the pAb-ELISA and 55.3 to 70.8% for the
489 mAb-ELISA. Coproantigen sensitivities strongly depend on the intensity of *E.*
490 *multilocularis* infection [13, 17–19], so foxes with low worm burdens are more likely
491 to result in false negatives. Knowing how highly aggregated distributed is *E.*
492 *multilocularis* in the fox population, it is likely that some foxes harboring low worm
493 burdens will be misclassified as negatives by this type of test. Overall, the best model

494 showed that the mAb-ELISA performed slightly better than the pAb-ELISA. Our
495 pAb-ELISA estimates are in line with a prior latent class study that included arecoline
496 purgation and egg-PCR in their analysis (SE_{dog} 55%, 95%CI 40.8 to 68.9% and SP_{dog}
497 70.6%, 95%CI 65.3 to 76.7%) [35], but lower than the originally test characteristics
498 reported (SE_{fox} ≈80%, SP_{fox} 95 to 99%) [13]. Often the coproantigen test has been
499 evaluated using the SCT as the gold standard test [13, 18] even though, as we have
500 discussed previously, its sensitivity is not perfect. Taking this into account the
501 coproantigen test's actual sensitivity in the field can be realistically considered to be
502 around 60% [6]. Furthermore, ELISA assays using polyclonal antibodies are prone to
503 batch-to-batch variation and thus their performance reproducibility cannot be
504 guaranteed. In this study however, sufficient quantities of polyclonal antibodies were
505 produced in one batch to allow 400,000 tests, which could be the basis of minimizing
506 this issue. In addition, the use of the polyclonal antibody test permitted the use of the
507 three or four-test models and thus was important to help define the parameters of the
508 other tests used, which do not suffer from this potential issue. Lastly, the estimates
509 obtained from the three and four-test models for the egg-PCR specificities ranged
510 between 85–98.2% and 87.3 to 99.1% and their sensitivities amid 47.4 to 61.0% and
511 48.5 to 61.0%. A field study in Kyrgyzstan also described the performance of this
512 multiplex PCR as a highly specific but low sensitive test (SE_{dog} 50%, 95%CI 29 to
513 72% and SP_{dog} 100%, 95%CI 97 to 100%) [49]. High specificities are expected
514 because the primers of this egg-PCR can identify and differentiate specifically the
515 *Echinococcus* parasitic egg-DNA found in the faeces, even though there is always the
516 possibility of false positive animals resulting from cross-contamination [50]. In
517 general, the PCR's sensitivity might be low under low worm burdens conditions or
518 the presence of juvenile worms (characteristic during pre-patent infections) [25].

519 Furthermore, during the DNA isolation procedure PCR-inhibitory substances could be
520 in the sample increasing the number of false negative results [25, 50].
521 In our models we considered conditional dependencies between sensitivities, but not
522 specificities. The absence of evident covariances among specificities can at least
523 partly be explained by the relatively high specificities and hence a low number of
524 false positives resulting in a too small sample size to realistically gain any information
525 for these covariances.
526 In both analyses, including the data of three and four tests, two covariates were
527 identified as significantly associated with *E. multilocularis* presence in the fox: *Age*
528 and concomitant infection with other c*estodes*. The incorporation of the effect of fox
529 age and the co-infection with other cestodes improved the goodness-of-fit of the
530 model to the data, and did not alter the estimation of the accuracy of the diagnostic
531 tests. Cestode species such as *E. multilocularis, Taenia* spp. *or Mesocestoides* spp.
532 have been found in the intestine of the red fox in Switzerland [46, 51]. Furthermore,
533 these tapeworms share with *E. multilocularis* the same species of rodents as
534 intermediate hosts (i.e. *Microtus arvalis, Arvicola terrestris*) [46, 51]. Preying on
535 rodents harboring diverse species of cestodes results in co-infections in the definitive
536 host. This supports the model finding of foxes with concomitant cestodes infection
537 presenting double the odds of harboring *E. multilocularis*. There are several studies
538 relating foxes of young age to *E. multilocularis* infection, although not always this
539 difference has been found statistically significant [46, 48, 52]. Several hypotheses
540 have been formulated to explain the frequent reporting of parasite infection and/or
541 burdens in juvenile foxes. One of the most suggested reasons behind these age-
542 differences is the potential existence of an acquired immunological response after
543 repeated infection [46, 48, 53]. However, other plausible causes such as differences in

their predatory or territorial behavior might result in juvenile animals with higher exposure to *E. multilocularis* infection compared to adults [54, 55]. A recent study modelling *E. multilocularis* abundance in Zurich foxes suggests that variations in infection pressure among age groups might be behind the observed differences in parasite loads between juveniles and adults [56]. Nevertheless, in our study the fox age was estimated on visual examination of teeth wear assessed by the researcher who was identifying the animals. Despite being a quick and easy method to distinguish between older and younger animals, it is also known to be less than 100% reliable as the teeth wear is subjected to individual characteristics such as type of diet or the occurrence of missing teeth [39, 57]. There is less evidence that sustains the potential association between *E. multilocularis* infection and sex of the fox [58]. Although young male foxes are known to expand their territory during the mating season [59] and thus, might have a higher risk of infection if, during their roaming behavior, they trespass clusters presenting an active parasite cycle with infected rodents. Nevertheless, the models did not find any significant differences in the odds of *E. multilocularis* infection between male and female foxes. This might be caused due to the small size of the study population or because of an unbalance of proportions in the data set, although the difference between numbers of collected males and females was not remarkable. Due to the small sample size, no internal validation was possible. Potentially, two sources of bias might have occurred. First, it could be that due to the sampling of the foxes during the hunting seasons a seasonal variation in cestode infection [56] might have introduced some sort of bias. Second, the PCR is designed to detect patent, but not pre-patent infections. With a life duration of 90 days and a third of this time being in a pre-patent state, the PCR results will never be unbiased in detecting all *E. multilocularis* infections [60].

For this analysis, uninformative as well as informative priors based on existing knowledge were used. By sensitivity analyses varying our prior information systematically, we found that our results are robust and are driven by the data and not by the prior information. Furthermore, the specificity of the necropsy and SCT was fixed to 100% [36]. In addition, the assumption of a high specificity in the identification of parasites by necropsy and SCT is supported by the lack of a potential differential diagnosis as, to the authors' knowledge, *E. granulosus* has not been yet found in foxes in Switzerland.

In the present study we wanted to assess the difference in determination of the cut-off by using two methods: the classical approach of considering the necropsy and SCT as a perfectly accurate test and the empirical method of deriving the ROC curve using the parameter estimations of the Bayesian latent class model. On this occasion, some differences were found, as the cut-off points obtained from the Bayesian methods were slightly higher than those obtained from the classical approach. To some extent, the use of the classic method of treating the necropsy and SCT results as true infection status to establish the coproantigen test accuracy could underestimate the specificity of the ELISA, in the case of having several necropsy and SCT false negatives. In addition, the building of the Bayesian ROC curves proved that the variation in the selection of the cut-off point for the ELISA did not affect the estimations of the other tests when including just one ELISA in the analysis. When including the two ELISAs the selection of the mAb-ELISA cut-off point did have an impact only on the pAb-ELISA estimations as the model structure accounted for conditional dependency between both coproantigen tests.

Finally, we employed the Bayesian latent class models to evaluate the test accuracy of the monoclonal ELISA to identify foxes presenting high parasite burdens of 100 or

more worms. The distribution of *E. multilocularis* in the fox population is highly aggregated with few animals making the largest contribution to the environmental contamination with parasitic eggs, and thus representing the majority of the zoonotic risk [21]. However, it is also possible that foxes with low worm burdens at the time of sampling could have had much higher burdens a short period before due to the dynamics of infection [60]. The highly infected foxes are believed to play a critical role in *E. multilocularis* transmission and ultimately human infection. Therefore, when monitoring this zoonotic parasite in the fox population it is paramount that surveillance programs employ diagnostic tests that can identify effectively foxes harbouring high parasite loads. The monoclonal coproantigen test proved to be a good tool for this purpose, showing high sensitivity and specificity to identify animals with moderate-to-high parasite burdens (≥100 worms). Furthermore, its good test performance along with its economic implementation and the fact that it can be performed on the faecal field samples without the need to collect dead animals, make this diagnostic test suitable for population studies in endemic areas.

However, in low prevalence and free areas where both a high sensitivity and a very high specificity (close to 100%) are needed, a confirmatory test is required. Although the MC-PCR fulfills these requirements [30], it has to be ensured that sufficient material from the fox scat will be available for both tests to be performed on the collected faecal samples. Otherwise the whole fox has to be collected and the ELISAs should be done on intestinal contents.

CONCLUSION

Through the implementation of Bayesian latent class models, we could estimate the true prevalence of infection and the specific performance of four diagnostic tests for

E. multilocularis on the study population. As we have seen, there is a lack of a gold standard test for *E. multilocularis* diagnosis in the definitive host. Furthermore, we know that the performance of these diagnostic techniques varies depending on the population investigated. Thus, the particular test performance on the population investigated has to be accounted for in order to be able to correctly interpret the diagnosis results [61]. The adoption of a Bayesian latent class approach helps to overcome the absence of a perfectly accurate test and therefore gives a more reliable indication of the tests performance to ensure that meaningful conclusions can be drawn. Furthermore, the flexibility inherent to this type of models allows the incorporation of the potential dependence between diagnostic tests and permits the investigation of the association of potential risk factors with true disease status [35, 49]. Finally, in the case of using a diagnostic test that needs the establishment of a cut-off point for the interpretation of its results, the Bayesian modelling facilitates the selection of this threshold value in a more reliable and comprehensive way than the classical method.

DECLARATIONS

ABBREVIATIONS

AE alveolar echinococcosis

DIC deviance information criterion

DNA Deoxyribonucleic acid

ELISA enzyme-linked immunosorbent assay

E. multilocularis Echinococcus multilocularis

mAb monoclonal antibodies

PCR polymerase chain reaction

ROC receiver operating characteristic

Se sensitivity

SCT Sedimentation and Counting Technique

SLT sedimentation counting technique

Sp Specificity

ETHICS APPROVAL

According the Swiss animal welfare legislation (English translation http://www.zuerchertierschutz.ch/fileadmin/user_upload/Tierschutzthemen/pdf/Tierschutzgesetz_e.pdf) Article 3, our study is not considered as animal experimentation. Therefore no ethical approval is needed. We received the confirmation via email that our study is not considered as animal experimentation Claudia Lawnitzak, Dr. med. vet. Amtliche Tierärztin, Tierschutz Tierversuche, Leiterin, Kanton Zürich, Gesundheitsdirektion, Veterinäramt, Zollstrasse 20, 8090 Zürich:

"Wir können Ihnen für den Kanton ZH bestätigen, dass es sich bei dem Forschungsprojekt um keinen Tierversuch gemäss Art. 3 TSchG handelt, da die Füchse nicht zu Versuchszwecken getötet wurden, sondern der primäre Verwendungszweck die Jagd zur Bestandeskontrolle war. Aus diesem Grund erfolgte auch keine Prüfung des Forschungsprojektes durch das Veterinäramt und durch die Tierversuchskommission."

CONSENT FOR PUBLICATION (mandatory): not applicable

AVAILABILITY OF DATA

The original data file with the diagnostic test results and information of covariates can be found in the additional file 1.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: BOA, SH, PRT and PD. Collection of data: MTAF, BOA and PD. Performed the experiments: MTAF, BOA and PD. Analyzed the data: BOA, SH and PRT. Wrote the paper: BOA, SH and PRT. All authors read and approved the final version of the manuscript

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FIGURE LEGENDS

Figure 1. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate, *Cestodes* for the best-fitting model to the results of three diagnostic tests.

Figure 2. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate *Age* for the best-fitting model to the results of three diagnostic tests.

Figure 3. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate, *Cestodes* for the best-fitting model to the results of four diagnostic tests.

Figure 4. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate *Age* for the best-fitting model to the results of four diagnostic tests.

Figure 5. Polyclonal ELISA ROC curves produced using the classical and the Bayesian approach.

Figure 6. Monoclonal ELISA ROC curves derived using the classical and the Bayesian approach.

Figure 7. Bayesian monoclonal ELISA ROC when the criteria to be positive by necropsy and SCT **is to present 100 or more *E. multilocularis.***

SUPPORTING INFORMATION

Additional file 1: Original Data file
(XLS)

Additional file 2: Bayesian latent-class model code for three diagnostic tests
(DOC)

Additional file 3: Bayesian latent-class model code for four diagnostic tests
(DOC)

Additional file 4: Description of the prior information used in the latent class models for three diagnostic tests
(DOC)

Additional file 5: Description of the prior information used in the latent class models for four diagnostic tests
(DOC)

Additional file 6: Sensitivity analysis for sensitivity of PCR
(PDF)

Additional file 7: Polyclonal ELISA ROC curves produced using the classical and the Bayesian approach (4 tests)

(TIF)

Additional file 8: Monoclonal ELISA ROC curves produced using the classical and the Bayesian approach (4 tests)

(TIF)

Additional file 9: Resulting covariances between sensitivities of the 3 and 4 test model

(DOC)

TABLES

Table 1. Observed percentages of collected foxes by age, sex and presence of cestodes co-infection.

		Sex		
		Female	Male	Total
Age	Young	21%	19%	40%
	Adult	25%	35%	60%
	Total	46%	54%	100%[a]
Cestodes	Yes	24%	36%	61%
	No	21%	18%	39%
	Total	46%	54%	100%[a]

[a] Total number of foxes = 300

Table 2. Parameters estimates (posterior means) with their corresponding 95% credibility intervals and the model goodness-of-fit to the data of the best model for three tests with and without covariates

	Model	Model with Age	Model with Cestodes	Model with Sex
SCT				
Se_1	0.92 (0.86 to 0.96)	0.91 (0.84 to 0.95)	0.91 (0.83 to 0.96)	0.91 (0.84 to 0.96)
Sp_1	1[a]	1[a]	1[a]	1[a]
Egg-PCR				
Se_2	0.54 (0.47 to 0.61)	0.54 (0.47 to 0.61)	0.53 (0.47 to 0.60)	0.54 (0.47 to 0.61)
Sp_2	0.92 (0.85 to 0.98)	0.92 (0.85 to 0.98)	0.91 (0.84 to 0.98)	0.92 (0.85 to 0.98)
pAb-ELISA				
Se_3	0.56 (0.48 to 0.64)	0.55 (0.47 to 0.63)	0.54 (0.46 to 0.63)	0.55 (0.47 to 0.63)
Sp_3	0.64 (0.54 to 0.73)	0.64 (0.53 to 0.74)	0.62 (0.51 to 0.72)	0.64 (0.53 to 0.73)
Prevalence	0.58 (0.53 to 0.65)	*NA*	*NA*	*NA*
$Cov = 1$[b]	*NA*	0.68 (0.58 to 0.78)	0.48 (0.38 to 0.59)	0.59 (0.49 to 0.69)
$Cov = 0$[b]	*NA*	0.55 (0.30 to 0.78)	0.69 (0.46 to 0.86)	0.61 (0.39 to 0.81)
Intercept	*NA*	0.76 (0.31 to 1.28)	-0.08 (-0.49 to 0.36)	0.35 (-0.05 to 0.80)
Slope (*OR*)	*NA*	0.56 (0.32 to 0.96)	2.36 (1.37 to 4.16)	1.12 (0.67 to 1.87)
DIC	1,129.2	1,126.7	1,120.4	1,130.9

SCT, Sedimentation and counting technique; *Se*, Sensitivity; *Sp,* Specificity; Egg-PCR, polymerase chain reaction; pAb-ELISA, polyclonal enzyme-linked immunosorbent assay (cut-off determined by considering necropsy and SCT as the gold-standard test); *NA*, Not applicable; *Cov*, Covariate; *OR*, Odds Ratio; *DIC*, deviance information criterion.

[a] Specificity of necropsy fixed to 1.

[b] Prevalence for respective covariate = 1 (adult, with other cestodes and male) and covariate = 0 (young, without other cestodes and female).

Table 3. Parameters estimates (posterior means) with their corresponding 95% credibility intervals and the model goodness-of-fit to the data of the best model for four tests with and without covariates

	Model	Model with Age	Model with Cestodes	Model with Sex
SCT				
Se_1	0.89 (0.83 to 0.93)	0.88 (0.82 to 0.93)	0.88 (0.81 to 0.93)	0.88 (0.81 to 0.93)
Sp_1	1[a]	1[a]	1[a]	1[a]
Egg-PCR				
Se_2	0.55 (0.49 to 0.61)	0.54 (0.48 to 0.61)	0.54 (0.48 to 0.61)	0.55 (0.48 to 60.8)
Sp_2	0.934 (0.87 to 0.99)	0.936 (0.87 to 0.99)	0.940 (0.87 to 0.99)	0.94 (0.874 to 0.99)
pAb-ELISA				
Se_3	0.56 (0.48 to 0.64)	0.56 (0.48 to 0.64)	0.55 (0.47 to 0.63)	0.56 (0.48 to 0.64)
Sp_3	0.66 (0.56 to 0.76)	0.66 (0.56 to 0.76)	0.65 (0.54 to 0.75)	0.66 (0.55 to 0.76)
mAb-ELISA				
Se_4	0.63 (0.55 to 0.71)	0.63 (0.55 to 0.71)	0.62 (0.54 to 0.70)	0.63 (0.55 to 0.71)
Sp_4	0.70 (0.60 to 0.79)	0.70 (0.60 to 0.80)	0.69 (0.59 to 0.79)	0.70 (0.60 to 0.80)
Prevalence	0.60 (0.43 to 0.66)	*NA*	*NA*	*NA*
$Cov = 1$[b]	*NA*	0.7	0.50	0.6

		(0.59 to 0.79)	(0.40 to 0.61)	(0.59 to 0.79)
Cov = 0[b]	*NA*	0.56 (0.31 to 0.78)	0.69 (0.46 to 0.86)	0.63 (0.31 to 0.78)
Intercept	*NA*	0.83 (0.38 to 1.34)	0.00 (-0.04 to 0.43)	0.39 (-0.01 to 0.83)
Slope (*OR*)	*NA*	0.55 (0.31 to 0.94)	2.24 (1.31 to 3.90)	1.16 (0.96 to 1.96)
DIC	1,507.0	1,501.9	1,497.2	1,506.2

SCT, Sedimentation and counting technique; *Se*, Sensitivity; *Sp,* Specificity; Egg-PCR, polymerase chain reaction; pAb-ELISA, polyclonal enzyme-linked immunosorbent assay; mAb-ELISA, monoclonal enzyme-linked immunosorbent assay (cut-off for both ELISAs determined by considering necropsy and SCT as the gold-standard test); *NA*, Not applicable; *Cov*, Covariate; *OR*, Odds Ratio; *DIC*, deviance information criterion.

[a] Specificity of necropsy fixed to 1.

[b] Prevalence for respective covariate = 1 (adult, with other cestodes and male) and covariate = 0 (young, without other cestodes and female).

Figure 1. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate, *Cestodes* for the best-fitting model to the results of three diagnostic tests

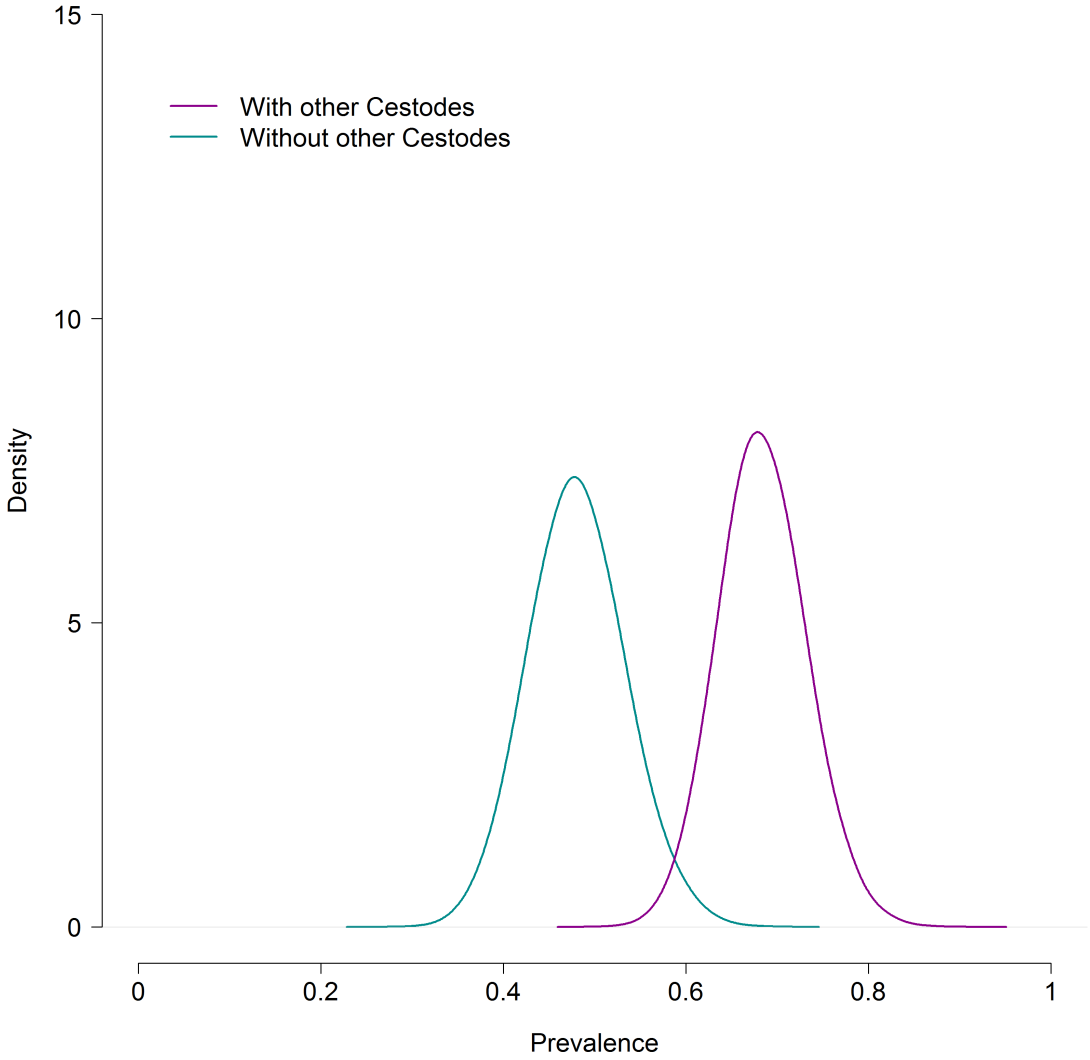


Figure 2. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate *Age* for the best-fitting model to the results of three diagnostic tests.

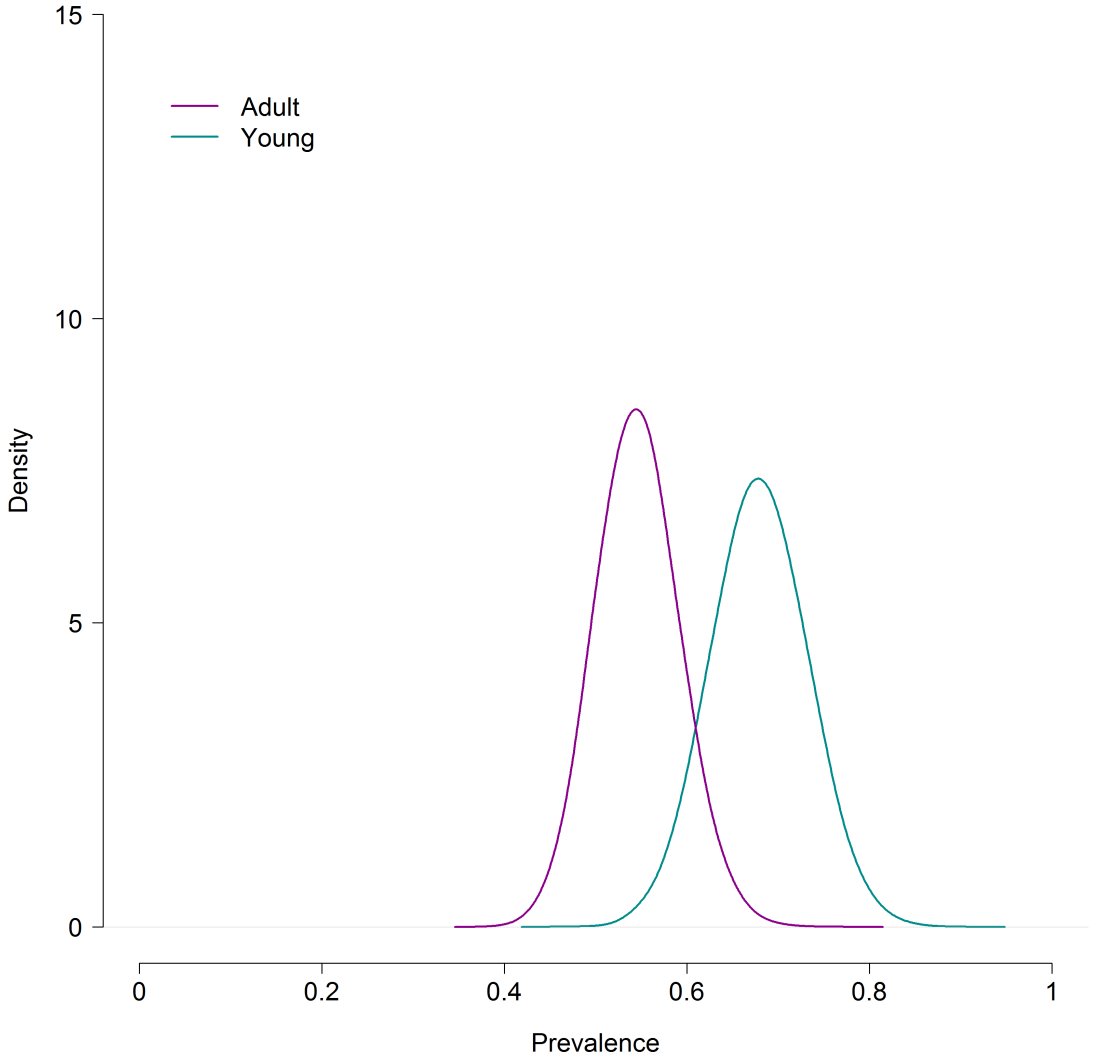


Figure 3. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate, *Cestodes* for the best-fitting model to the results of four diagnostic tests.

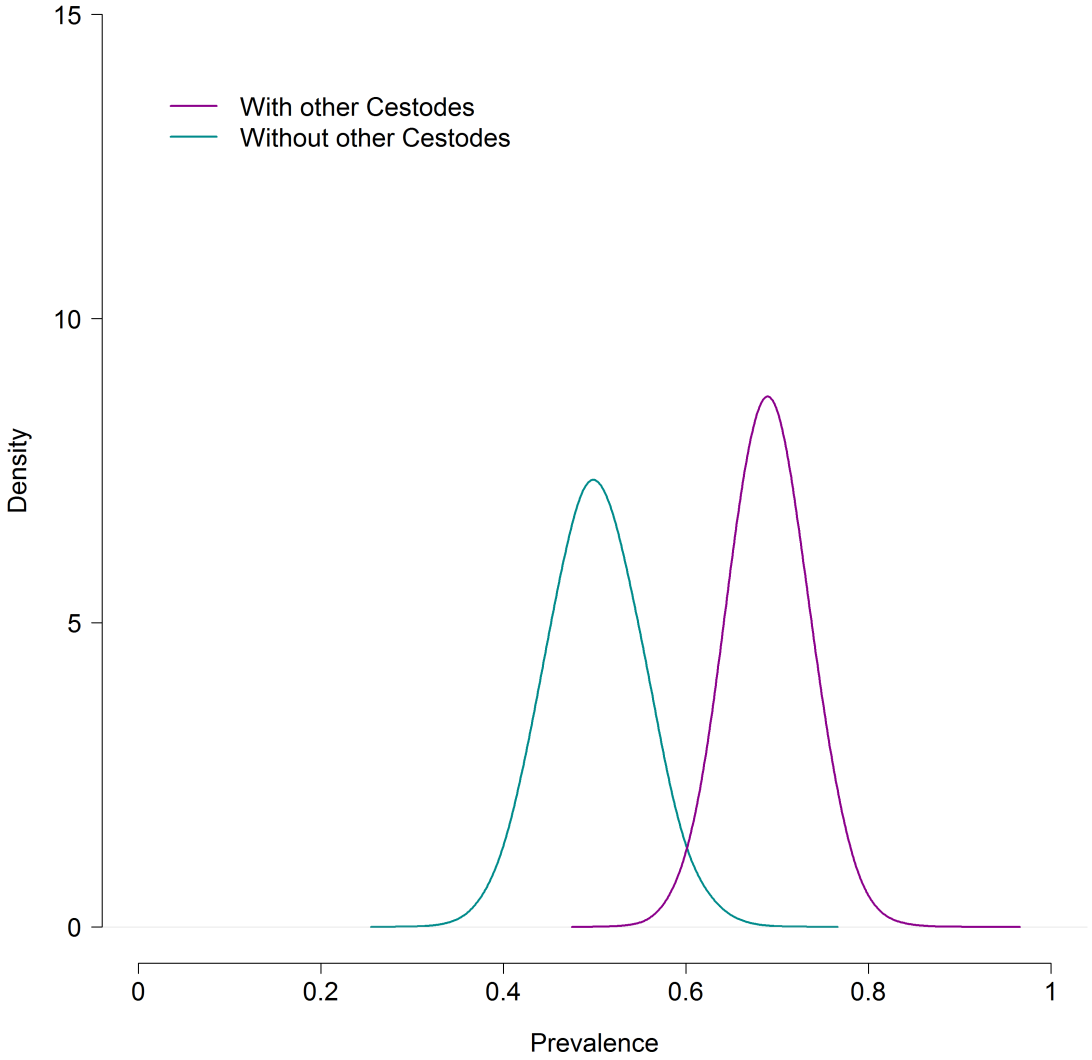


Figure 4. Posterior distribution of *E. multilocularis* prevalence in foxes with and without the significant covariate *Age* for the best-fitting model to the results of four diagnostic tests.

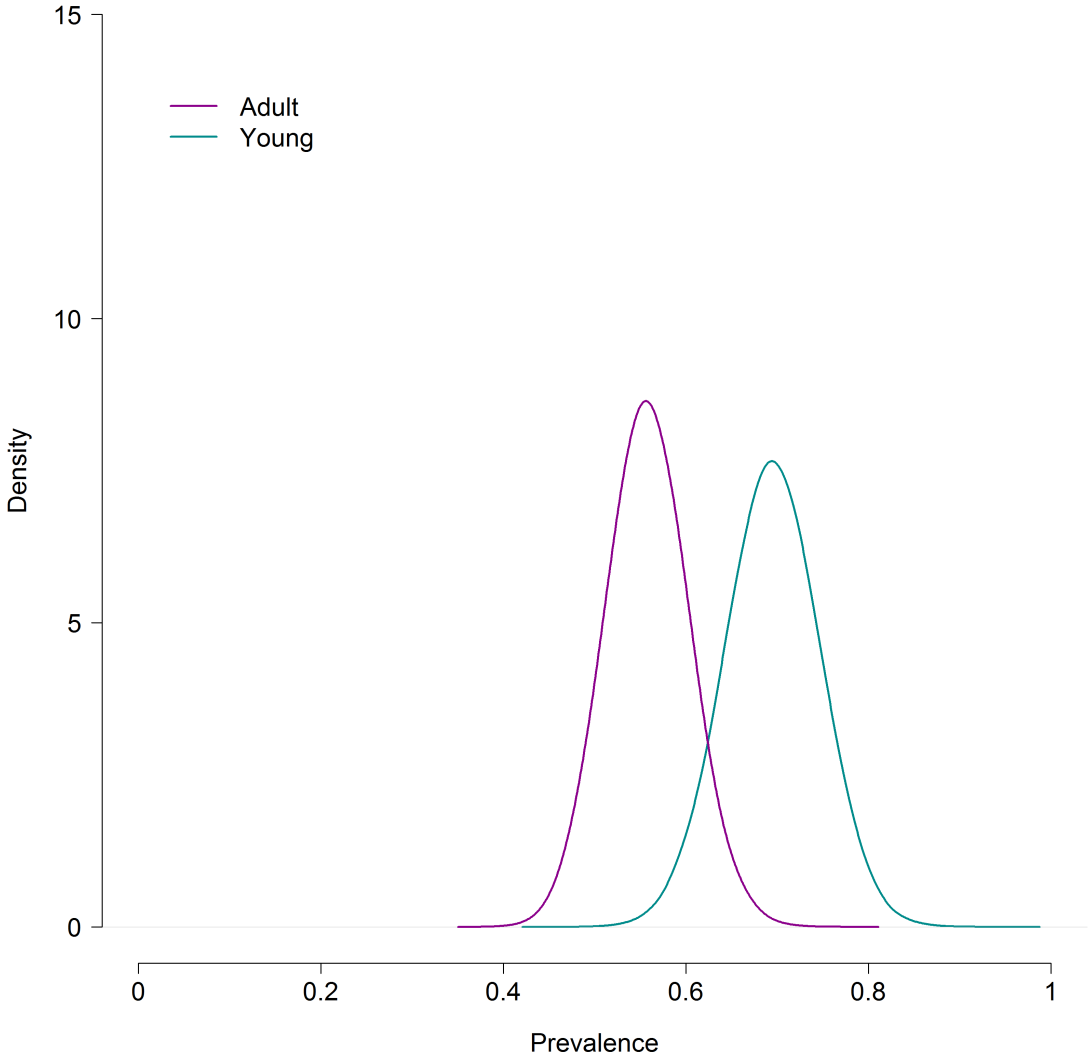


Figure 5. Polyclonal ELISA ROC curves produced using the classical and the Bayesian approach.

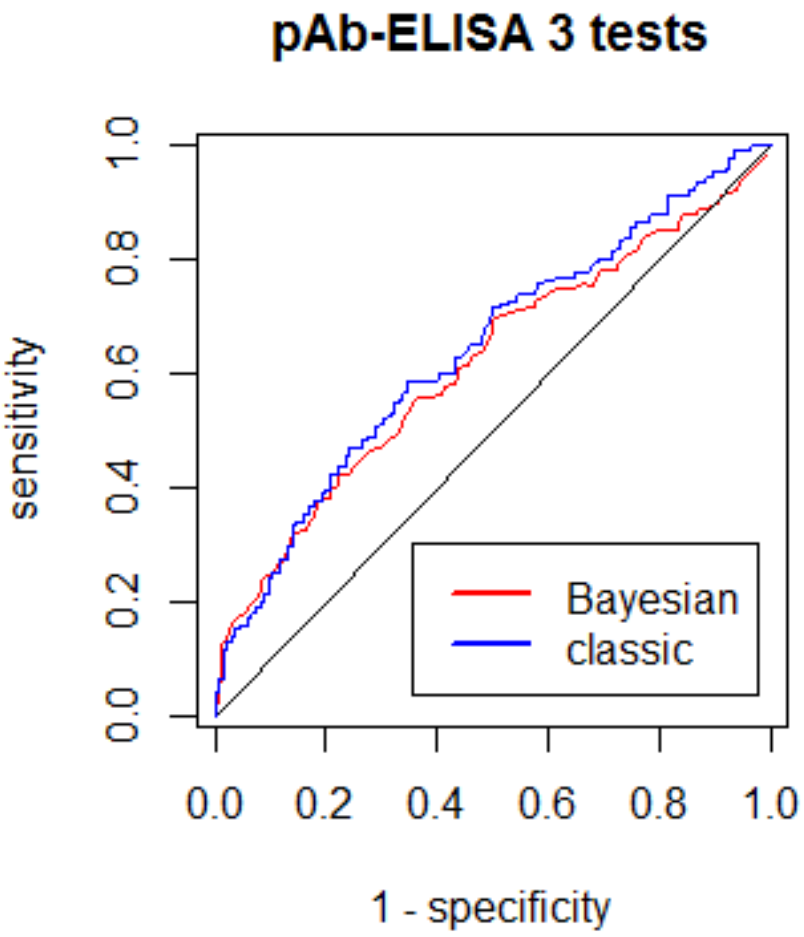


Figure 6. Monoclonal ELISA ROC curves derived using the classical and the Bayesian approach.

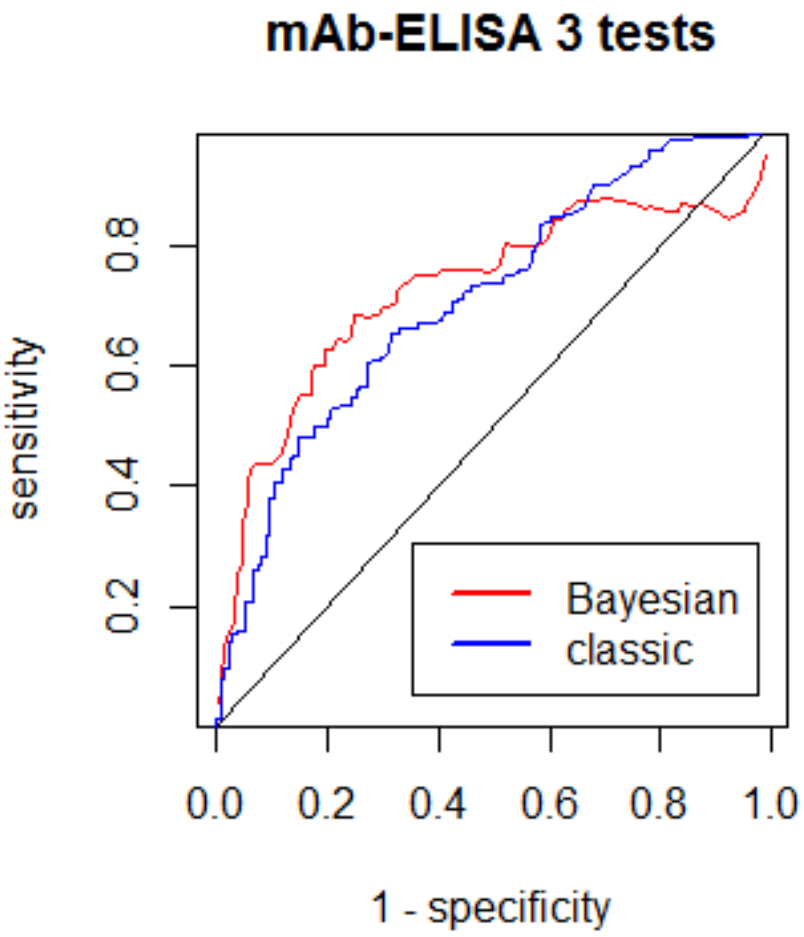
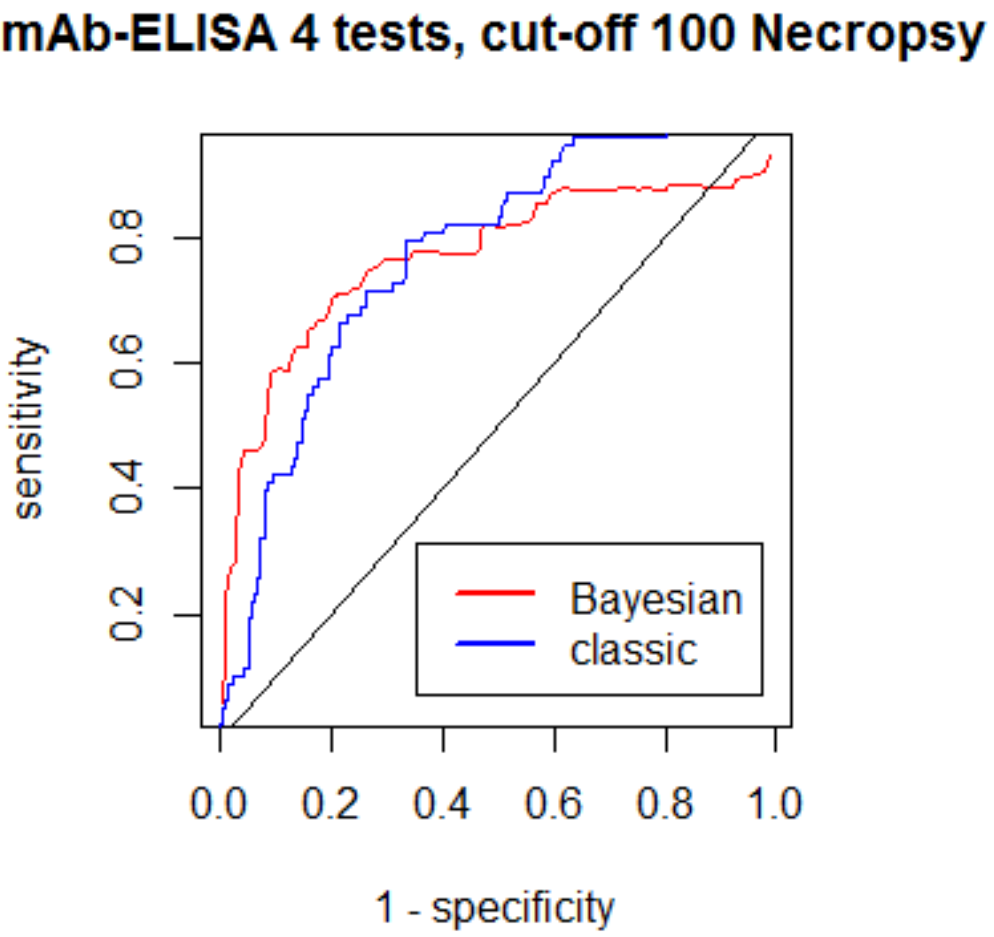


Figure 7. Bayesian monoclonal ELISA ROC when the criteria to be positive by necropsy and SCT is to present 100 or more *E. multilocularis*.



Synthesis, discussion and conclusion

Synthesis

The scope of the present thesis is to broaden the current epidemiology's knowledge on *E. multilocularis* infection in foxes through the building and testing of mathematical models to field data from a high prevalence country as it is Switzerland [1]. The work resulted from the completion of four main studies collectively constitute this thesis. Paper 1 presents a comprehensive summary of significant determinants for animal echinococcosis, caused by *E. granulosus* and *E. multilocularis*. The outcome of this first study provided with sound background information on the currently known risk factors for echinococcus infection in animals, such are host age, host gender, certain weather conditions or the type of host habitat. In Paper 2, parasite prevalence models were adapted to the Zurich foxes' situation to test hypotheses on some of the risk factors resulted from the first study. This second study reports evidence of variations in the number of parasite insults experienced by fox per year across urban habitats and yearly seasons, within the municipality of Zurich. Zurich foxes experienced the highest number of parasite insults during the cold months of the year. In addition, foxes from the periphery of the city were exposed more frequently to parasite infection compared to foxes from the urban centre. No evidence of parasite-induced immunity was found. However, due to the clumped nature of *E. multilocularis*' distribution in foxes, parasite burdens represents a more influencing epidemiological parameter when assessing the infection risk in humans. In Paper 3, parasite abundance models were adapted to evaluate the influence of some risk factors on the number of parasites developing in the Zurich fox after an infectious insult per year. This third study also suggested variations in parasites harboured by foxes across urban habitats and yearly seasons, presenting greater parasite burdens during the winter. Only in the periphery of the city, evidence of age-based differences in parasite burdens were identified although no evidence of parasite-induced immunity was found. Presently there is a lack of a 100% accurate test to identify *E. multilocularis*' infection in foxes. With the implementation of Bayesian latent class models the uncertainty inherent to the diagnosis results can be acknowledged, making the modelling estimates more reliable. In Paper 4, the results derived from four types of the most frequently employed tests for *E. multilocularis*' diagnosis were fitted to latent class models. With this fourth study the diagnosis tests

characteristics and the true *E. multilocularis* prevalence, which was associated with the presence of concurrent cestode infection in the fox and with being a young animal, were estimated. In addition this type of model served as a platform for the assessment of the potential impact on the tests' performance depending on the chosen method for the cut-off point selection of the ELISAs tests.

- **Risk factor systematic review**

The review of the determinants of parasite infection in the animal host provides with a foundation for the formulation of hypotheses, which can be after explored within the mathematical framework. To that purpose, and since to the authors' knowledge there was research gap on this topic, Paper 1 presents a systematic review to address the first research question of the present thesis: *What are the risk factors for Echinococcus infection in animal hosts that have been determined through the employment of associative models?*

E. granulosus transmission is mainly supported by the dog-sheep cycle and, consequently close contact with dogs and livestock are among the most reported significant factors associated with an increased risk of cystic echinococcosis (CE) in humans. Hence, CE's prevention measures should especially be aimed in tackling infection determinants in animal hosts, such as impeding free-range dogs, avoiding their feeding with raw offal, giving them regular anthelmintic treatment and improving hygiene at abattoirs along with the banning of home-slaughtering. Such measures are greatly linked with individual behaviour and the level of knowledge regarding this disease; thus, educational campaigns should be included in order to reduce infection risk in humans. In other hand, *E. multilocularis* infection in the animal hosts is associated with a complex set of individual and ecological factors that influence the viability of parasitic eggs in the environment, animal host population dynamics and their predator-prey relationship. In Europe, *E. multilocularis*' transmission is primarily supported by the fox-rodent cycle. Hence, an increased risk of alveolar echinococcosis (AE) in humans relies on the closeness from an actively parasite cycle along with the subsequent greater exposure to infective eggs. AE's prevention measures should lower the contamination with parasitic eggs through the

control of the animal host species and the regular deworming of definitive hosts [2]. However, the deliver of information campaigns that increase public awareness towards AE is crucial in lessening the transmission to humans [2].

‣ Mathematical modelling of parasite prevalence

Several hypotheses of the spatio-temporal variations in the force of infection (FOI) - defined as *E. multilocularis* exposure experienced by the Zurich fox per year- and on the potential presence of parasite-induced immunity were assessed through parasite prevalence models. Paper 2 addresses the second group of research questions of the present thesis: *What is the yearly rate at which susceptible foxes are challenged with Echinococcus infection within the municipality of Zurich? Does it vary across seasons and habitat types? Is the hypothesis of existing parasite-induced immunity supported by the data?*

Field data supported the existence of a periodic FOI with spatial differences across different urbanization zones in Zurich but gave no evidence for the presence of host-induced immunity. Through the characterization of the FOI a more cost-effective control plan could be planned. Parasite control strategies are suggested to strength their efforts during the winter within the periurban zone of Zurich which mainly consisted of green areas frequently used for recreational activities [3]. Even though foxes are able to move between habitats within the city without restrictions, previous studies have shown that Zurich foxes display relatively small home range sizes [4] and differences in parasite prevalence have been reported between minor distances [5]. Nevertheless, the FOI quantified in the second study only reflects fox exposure to infection but not the actual number of parasites that would develop after every parasite insult. Parasite loads in the definitive host represents a crucial parameter in *E. multilocularis* epidemiology as heavily infected foxes can excrete high numbers of infective eggs contributing greatly to environmental contamination and, ultimately increasing the risk of human infection [6,7]. Therefore, further research on parasite abundance in the fox is needed to characterize the infection risk for AE in a more reliable way.

- **Mathematical modelling of parasite abundance**

Several hypotheses of the spatio-temporal variations in the infection pressure (IP) - number of *E. multilocularis* that would establish after each parasite exposure experienced by the Zurich fox in a year- and on the presence of parasite-induced immunity were assessed through parasite abundance models. Paper 3 addresses the third group of research questions of the present thesis: *What is the annual number of parasites that developed in foxes after being challenged with Echinococcus infection within the municipality of Zurich? Are there any differences across seasons and habitat types? Is there an age-dependent distribution of worms? Is the hypothesis of existing parasite-induced immunity supported by the data?*

The data on parasite burdens supported the absence of host immunity but acknowledged a decreasing infection pressure with the increasing fox age, only in the periurban zone. In addition, the abundance model concurred with the existence of variations in the infection pressure in Zurich foxes among urbanisation zones and among seasons, with the highest peaks occurring during autumn and winter. The results derived from this analysis reiterate on the recommendation that parasite control efforts should be reinforced during the cold seasons in the periurban zone of the city in order to lessen the parasite burdens in Zurich foxes and, consequently, the environmental contamination with infective eggs. Yet a probably less demanding anthelmintic baiting strategy should also be implemented in the urban centre to address the sporadic occurrence of heavily infected foxes from adjacent areas wandering the city centre, as model predictions indicated that urban foxes can develop up to 8,300 parasites per infection event. Parasite prevalence and abundance data modelled in the second and third study derived from the results of imperfectly accurate diagnostic tests. Hence, the next step is to acknowledge the inherent uncertainty related to the data that feeds the models.

- **Latent class analysis of diagnostic test results on parasite infection**

The absence of a gold standard test for the identification of *E. multilocularis* infection in foxes poses a major constrain in the correct interpretation of parasite monitoring in wildlife and of the results from transmission models that rely on such tests. In the

fourth study, data resulting from the performing of four types of imperfect diagnostic tests for the presence/absence of *E. multilocularis* in Swiss foxes were fitted to latent class models. The flexibility in the structure of this type of model allowed the evaluation of some infection determinants plus the building of receiver operating characteristic (ROC) curves for the ELISA tests. Paper 4 addresses the fourth group of research questions of the present thesis: *What is the true E. multilocularis prevalence in foxes from the midland regions of Switzerland after accounting for the uncertainty related to the currently available diagnostic tests? What are the characteristics of these tests on the study population? Can we determine any associations between infection status and the information gathered on potential risk factors? Is there any difference in the establishment of the optimal cut-off for the ELISA test if a more empirical method is used instead of considering the necropsy and the SCT as the gold standard test?*

The outcome of this analysis gave the estimations of the sensitivities and specificities of four diagnostic tests. Through the building of ROC curves it was confirmed that the establishment of the cut-off point for the ELISAs tests using a Bayesian empirical approach instead of the classic method (considering necropsy as the gold standard) did not bring meaningful differences to the model estimates. The analysis determines the *true* prevalence of *E. multilocularis* infection in Swiss foxes, which was associated with the presence of concurrent cestode infection and with being a young animal. The estimation of the true parasite prevalence is paramount as establishes the baseline against to which assess the effectiveness of any control programme in the definitive host [8]. Furthermore, the model estimations of the tests' characteristics can be incorporated in future parasite transmission models to obtain more meaningful conclusions. Lastly, by accompanying the parasite monitoring results with information on the real test characteristics, the results can be evaluated and compared to other monitoring studies in a more transparent manner.

‣ Conclusion

Human echinococcosis remains a severe zoonosis representing an important cause of ill health and economic burden that falls particularly heavily on low-income

communities. Veterinary epidemiologists are in a leading position to increase the scientific understanding of transmission of this disease to animals, which is essential for effective prevention and control planning. To that purpose the objective of the present thesis is to increase the current knowledge of *E. multilocularis* epidemiology in the animal host so it can be of use to the design and implementation of measures to control this parasite in the animal reservoirs that support its life cycle. The present research provides with an insight on the risk factors for parasite infection in the animal hosts, a more specific understanding on the spatio-temporal variations on parasite infection and biomass in foxes collected in Zurich and the estimation of *true E. multilocularis* prevalence and test characteristics of four of the most employed diagnostic tests in foxes. Future steps in modelling parasite dynamics could concentrate in the further development of mathematic techniques to better represent the clumped nature of the infection process and to be able to incorporate, in a more comprehensive way, the temporal and spatial factors affecting *E. multilocularis* transmission, while using true infection status as the diagnostic uncertainty inherent in the test results should be acknowledged.

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Appendix: Supporting information

Appendix: Supporting information-Paper 1

Checklist S1: PRISMA Checklist

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7-8
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6-7

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7-8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	NA
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8-17
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7-8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8-17
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	NA
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	7-8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	NA
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	25
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	7
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	25-26
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	26

Table S1: Glossary of statistical terms

Table S1. Glossary of statistical terms

Term	Definition
Akaike information criterion (AIC)	A measure of the relative goodness of fit of a statistical model. The second-order Akaike Information Criterion (AICc) is equivalent to AIC but with a correction for finite sample sizes. The Akaike difference Delta (ΔAICc) shows the difference between the model AICc and the lowest AICc for the model set. The AICc weights help to valuate the relative importance of each variable. Lower AIC/AICc, lower ΔAICc or higher AICc weight indicate good plausible models and viceversa
Analysis of variance	Statistical technique which assesses the effect of categorical explanatory variables on the response variable
Binomial logistic regression	Statistical method to analyse the association between a binomial response variable (such as presence/absence of infection) and one or more explanatory variables. In such logistic regression the response variable is transformed to the logistic scale
Coefficient of determination (R^2)	Proportion of variation in the response variable explained by the statistical model
Conditional autoregressive spatial modelling	Type of regression model that captures spatial dependency and provides information on spatial relationships among the variables modeled
Conditional logistic regression	Type of logistic regression that analyses a categorical response variable when observations are not independent but matched or grouped in some way
Confidence intervals (CI)	Intervals that provide a range of values for a variable of interest constructed so that if the experiment can be repeated many times, the value of the parameter will lie within this interval in a confidence level of occasions, usually set to 95%
Correlation coefficient	Determines the degree of linear relationship between two variables
Cross-sectional	Epidemiologic study involving the observation of a population, or a

Table S1: Glossary of statistical terms (cont.)

study	representative subset, at one specific point in time
Determinant	Any factor or variable that can affect the frequency with which a disease occurs in a population
Explanatory variable	A factor or exposure that may influence the occurrence of the response variable
Fixed-effects model	Type of model that treats the varying coefficients of explanatory variables as if the quantities were non-random (constant over all groups)
Interaction effect	Situation when the effect of the explanatory variable(s) on the response variable depends on the value of another explanatory variable(s)
Linear regression	Estimates the best-fitting straight line to describe the linear relationship between two variables
Mixed-effects model	Type of multilevel model that includes both fixed and random effects
Multilevel models	Statistic models of parameters that vary at more than one level
Multivariable analysis	Statistic analysis that includes more than one explanatory variable and where any potential issues, such confounding and interaction, are taken into account
Multivariate analysis	Statistical analysis where there is more than one response variable regardless of the number of explanatory variables included
Negative binomial regression	Type of statistical model that assumes that the dependent variable has an aggregated distribution described by the negative binomial distribution
Odds ratio (OR)	Measure of association between exposure and disease. The OR represents the odds that disease will occur given a particular exposure, compared to the odds of disease occurring in the absence of it
Ordinal logistic regression	Type of logistic regression that analyses an ordinal categorical response variable (with categories that have some intrinsic order) with one or more explanatory variables
p -value	Probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true
Random-effects model	Type of multilevel model which varying coefficients of explanatory variables have a different value for each of the groups (or levels)

Table S1: Glossary of statistical terms (cont.)

Response variable	Variable that is the focus of the analysis, whose variation is trying to be explained by the explanatory variables
Risk ratio (RR)	Measure of association between exposure and disease. The RR gives a ratio of disease risk in the exposed compared to disease risk in the non-exposed
Semivariogram	Function describing the degree of spatial dependence of a spatial random field or stochastic process
Spatial scan statistic	Statistical method to test for spatial clustering
Univariable analysis	Statistic analysis that includes just one explanatory variable
Univariate analysis	Statistic analysis that deals with a single response variable regardless of the number of explanatory variables included
Variable	Characteristic that can be measured in different individuals or groups capable of adopting different values
Zero inflated model	Type of count regression model when there are additional zero counts then can be described by a standard count model such as Poisson or negative binomial

Table S2: Studies assessing association between *E. granulosus* infection in dogs and potential access to raw offal

Table S3. Studies assessing association between *E. granulosus* infection in dogs and potential access to raw offal

Reference	Study Information	Statistical Method	Significant Factor
Bchir et al., 1987 [29]	Post-mortem inspection of 50 dogs in Central Tunisia	Univariable analysis	Dogs shot within 1 km of a refuse dump (p<0.001)
Parada et al., 1995 [27]	Arecoline purgation of 704 dogs in Durazno (Uruguay)	Univariable analysis	Dogs with access to fields (p<0.05) and not tied-up dogs (p<0.001)
Moro et al., 1999 [20]	Arecoline purgation of 63 dogs in central Peruvian Andes	Univariable analysis	Dog fed with hydatid-infected viscera were (p<0.004) and sheep-dogs were more likely to be infected (p<0.001)
Wang et al., 2001 [23]	Coproantigen examination of 139 owned dogs in Narenhebuke commune (China)	Univariable analysis	Dogs from the winter area presented higher coproantigen positivity than the ones from the summer pasture (p<0.01)
Shaikenov et al., 2003 [25]	Arecoline purgation of 2,071 dogs in Southern Oblasts (Kazakhstan)	Univariable analysis	Farm dogs present higher abundance of infection and prevalence (p<0.001)
Buishi et al., 2005 [24]	Coproantigen examination of 334 dogs in Tripoli (Libya)	Multivariable logistic regression	Sheep-dogs showed an increased risk of coproantigen positivity compared to household dogs (OR 9.791, 95%CI 1.081-88.66, p=0.042)
Buishi et al., 2005 [33]	Coproantigen examination of 1,164 farm dogs in Wales (UK)	Multivariable logistic regression	Unrestrained dogs had higher risk of coproantigen positivity (OR 2.91, 95%CI 1.77-4.8, p<0.0001)
Perez et al., 2006 [26]	Coproantigen examination of 748 dog faecal samples from livestock farms in Rio Negro (Argentina)	Univariable analysis	Dog prevalence increased with increasing number of dogs (p=0.0028) (i.e. OR 4.19, comparing having 1 dog with having ≥5) and sheep

Table S2: Studies assessing association between *E. granulosus* infection in dogs and potential access to raw offal (cont.)

			(p=0.0039) (i.e. OR 4.29, compared 0 with ≥ 2,501)
Buishi et al., 2006 [21]	Coproantigen examination of 161 dogs in Turkana (Kenya)	Multivariable logistic regression	Dogs fed with raw offal (OR 22.74, 95%CI 2.60-199.08, p=0.005) and dogs free to roam were at higher risk of being coproantigen positive (OR 14.56, 95%CI 2.70-78.50, p=0.002) whereas the proper disposal of carcases by dog-owners reduced such risk (OR 0.07, 95%CI 0.01-0.33, p=0.001)
El Shazly et al., 2007 [28]	Post mortem examination of 540 dogs in Dakahlia (Egypt)	Univariable analysis	Rural dogs compared to urban dogs (p=0.03)
Guzel et al., 2008 [30]	Coproantigen examination of 79 owned dogs in Antakya (Turkey)	Univariable analysis	Unrestrained dogs had increased risk of coproantigen positivity (p<0.05)
Huang et al., 2008 [31]	Coproantigen examination of 23 stray dogs and 580 owned dogs in Tibet (China)	Univariable analysis	Unrestrained dogs (p<0.01) compared to those tied during the day or/and night
Inangolet et al., 2010 [34]	Post mortem examination of 327 dogs in the Moroto District (Uganda)	Ordinal logistic regression	Stray dogs presented higher parasite burdens compared to domesticated dogs (OR 5.42, 95%CI 2.27–12.92), p<0.001)
Acosta-Jamett et al., 2010 [22]	Coproantigen examination of 334 dogs in Coquimbo (Chile)	Multivariable mixed-effects logistic regression	Dogs from households not practising home-slaughter (OR 0.04, 90%CI 0.01–0.13, p=0.001), from rural sites (OR 0.01, 90%CI 0.002–0.05, p=0.001) and with longer

Table S2: Studies assessing association between *E. granulosus* infection in dogs and potential access to raw offal (cont.)

			distance to rural areas (OR 0.01, 90%CI 0.001–0.17, p=0.007) showed lower prevalence
Mastin et al., 2011 [32]	Coproantigen examination of 577 dogs in South Powys (Wales)	Multivariable mixed-effects logistic regression	Dogs regularly roaming had higher coproantigen positivity (OR 4.93, 95%CI 1.87–13.00, p=0.001)

Measures of association reported when available
Abbreviations: OR, odds ratio; CI, confidence interval.

Table S3: Studies identifying significant associations of age/gender and infection of dogs with *E. granulosus*

Table S3. Studies identifying significant associations of age/gender and infection of dogs with *E. granulosus*

Reference	Study Information	Statistical Method	Significant Factor
Parada et al., 1995 [27]	Arecoline purgation of 704 dogs in Durazno (Uruguay)	Univariable analysis	Male dogs were more likely to be infected than females (p<0.05)
Sharifi et al., 1996 [35]	Post mortem examination of 6,500 dogs in Kerman (Iran)	Univariable analysis	Dogs from 0-2 years showed higher prevalence compared to dogs of 3-4 years (p<0.05) and >5 years (p<0.002)
Buishi et al., 2005 [24]	Coproantigen examination of 334 dogs in Tripoli (Libya)	Multivariable logistic regression	Dogs >5 years presented lower coproantigen positivity (OR 0.852, 95%CI 0.731-0.993, p<0.04)
Buishi et al., 2006 [21]	Coproantigen examination of 161 dogs in Turkana (Kenya)	Multivariable logistic regression	Dogs ≤5 years presented higher coproantigen-positive results (OR 0.47, 95% CI 0.29-0.76, p=0.002)
Acosta-Jamett et al., 2010 [22]	Coproantigen examination of 334 dogs in Coquimbo (Chile)	Multivariable mixed-effects logistic regression	Dogs > 2 years presented lower odds of being coproantigen-positive (OR 0.11, 90% CI 0.04–0.29, p=0.001)
Inangolet et al., 2010 [34]	Post mortem examination of 327 dogs in the Moroto District (Uganda)	Ordinal logistic regression	Dogs >5 years presented lower worm counts (OR 0.07, 95%CI 0.04–0.16, p<0.001)

Measures of association reported when available
Abbreviations: OR, odds ratio; CI, confidence interval.

Table S4: Studies assessing association between *E. granulosus* infection in dogs and socio-economic factors

Table S4. Studies assessing association between *E. granulosus* infection in dogs and socio-economic factors

Reference	Study Information	Statistical Method	Significant Factor
Burridge et al., 1977 [37]	Arecoline purgation of dogs to assess progress of hydatid control in New Zealand	Multivariable regression	Percentage of Maori people was negatively related with rate of progress in disease control (R^2=0.215) [1]
Pappaioanou et al., 1984 [36]	Arecoline purgation of dogs to assess progress of anti-echinococcosis campaign in Cyprus	Multivariable logistic regression	Ethnic village type was associated with higher dog prevalence (p<0.0001)
Parada et al., 1995 [27]	Arecoline purgation of 704 dogs in Durazno (Uruguay)	Univariable analysis	Dogs reported to be dewormed with praziquantel were less likely to be infected (p<0.01)
Buishi et al., 2005 [33]	Coproantigen examination of 1,164 farm dogs in Wales (UK)	Multivariable logistic regression	Dogs not dewormed frequently (>6 months) presented higher risk of coproantigen positivity (OR 3.16, 95%CI 1.46–6.85, p=0.004)
Buishi et al., 2005 [24]	Coproantigen examination of 334 dogs in Tripoli (Libya)	Multivariable logistic regression	Dog owners reporting lack of knowledge about hydatid disease presented an increased risk of having a positive coproantigen dog (OR 3.278, 95%CI 1.045-10.28, p=0.042)
Huang et al., 2008 [32]	Coproantigen examination of 23 stray dogs and 580 owned dogs in Tibet (China)	Univariable analysis	Dogs whose owners lacked hydatid transmission knowledge (p<0.05) and did not have de-worming practice (p<0.01)
Acosta-Jamett et al., 2010 [22]	Coproantigen examination of 334 dogs in Coquimbo (Chile)	Multivariable mixed-effects logistic regression	Households reporting not have been dewormed their dogs in the last 2 months presented higher prevalence (OR 5.23, 90%CI 1.98–13.8, p=0.005)

Measures of association reported when available

[1] The percentage of Maori people in the total population explained 21.5% of the variation in *E. granulosus* infection in dogs. No significant test is provided for the R^2 change.

Abbreviations: R^2, Coefficient of determination; OR, odds ratio; CI, confidence interval.

Table S5: Associative studies of *E. granulosus* infection in intermediate hosts

Table S5. Associative studies of *E. granulosus* infection in intermediate hosts

Reference	Study Information	Statistical Method	Significant Factor
Pandey et al., 1986 [53]	Abattoir survey of 2,246 sheep, 510 goats and 35 dromedaries in Morocco	Univariable analysis	Age increasing prevalence in sheep (p<0.01)
Abdul-Salam et al., 1988 [60]	Parasitological examination of 293 camels in Kuwait	Univariable analysis	Females presented higher cyst infection (p=0.015) compared to males
Ming et al., 1992 [62]	Parasitological examination of 2,106 sheep in the Xinjiang Uygur Autonomous Region (China)	Univariable analysis	Females presented higher cyst prevalence (p<0.0001)
Cabrera et al., 1995 [58]	Post-mortem examination of 501 sheep in Florida (Uruguay)	Univariable analysis	Difference between age groups in sheep (p<0.01)
Njoroge et al., 2002 [38]	Abattoir survey of 381 cattle, 588 sheep, 5,752 goats and 70 camels in Turkana (Kenya)	Univariable analysis	Geographic location (p<0.05)
Tashani et al., 2002 [54]	Abattoir survey of 614 cattle, 1087 sheep, 881 goats and 428 camels in Benghazi (Libya)	Univariable analysis	Age and prevalence correlated in sheep (p=0.042), cattle (p<0.001) and camels (p=0.053). Sheep had the highest hydatid infection (p<0.01) and goats the lowest (p<0.01). Higher prevalence in females than males (p<0.01)
Umur et al., 2003 [55]	Abattoir survey of 1,355 cattle, 218 sheep and 104 goats in Burdur (Turkey)	Univariable analysis	The prevalence and number of cysts increased with age (p<0.05)
Islam et al., 2003 [49]	Abattoir survey of 405 cattle, 142 sheep, 292 goats and 108 buffaloes in Cox's Bazar (Bangladesh)	Univariable analysis	Host species and age (p<0.001)
Sharma et al., 2004 [68]	Parasitological examination of 236 pig meat samples in	Univariable analysis	Higher prevalence in pigs reared in extensive conditions

Table S5: Associative studies of *E. granulosus* infection in intermediate hosts (cont.)

	Punjab (India)		compared to intensive production (p<0.05)
Ahmadi et al., 2005 [39]	Abattoir survey of 661 camels in Iran	Univariable analysis	Geographic location (p<0.005)
Ansari-Lari, 2005 [46]	Retrospective abattoir study of 131,716 cattle, 577,090 sheep and 135,233 goats in Shiraz (Iran)	Univariable analysis	Seasonal variations in prevalence (p<0.0001)
Scala et al., 2006 [52]	Abattoir survey of 771 sheep in Sardinia (Italy)	Multivariable logistic regression	Cyst infection increased with host age (OR 1.15, 95%CI 1.0736– 1.2478, p<0.0001)
Azlaf et al., 2006 [40]	Abattoir survey of 618 cattle, 2,948 sheep, 2,337 goats, 482 camels and 455 equines in Morocco	Univariable analysis	Geographic origin and host species (p<0.0001)
Banks et al., 2006 [41]	Abattoir survey and retrospective abattoir data of 32,567 cattle in Queensland (Australia)	Multivariable logistic regression	Geographic origin and age (p<0.01)
Daryani et al., 2007 [61]	Abattoir survey of 928 cattle, 3,765 sheep, 445 goats and 243 buffaloes in Ardabil (Iran)	Univariate analysis	Female gender (sheep and cattle) (p<0.001) and seasonal prevalence patterns (sheep) (p<0.001)
Cringoli et al., 2007 [64]	Abattoir survey of 2587 cattle and 612 water buffaloes in the Campania (Italy)	Univariable analysis	Host species and sheep farms closer to cattle positive farms than water buffalo positive farms (p<0.001)
Lahmar et al., 2007 [43]	Ultrasound screening of 1,039 sheep in the northeast of Tunisia	Univariable analysis	Geographic origin (p<0.01) and age (p<0.05)
Christodoulopoulos et al., 2008 [56]	Abattoir survey of 700 hoggets and 1500 sheep in Thessaly (Greece)	Univariable analysis	Age (p<0.001)
Ernest et al., 2009 [45]	Retrospective abattoir study of 2,677 cattle and 607 sheep and 3,047 goats in Arusha (Tanzania)	Univariable analysis	Host species (p<0.001) and geographic location (p<0.001)
Bruzinskaite et al., 2009 [50]	Abattoir of 648 pigs in Southwestern (Lithuania)	Univariable analysis	Age (p<0.01) and type of farm (p<0.02)
Nonga et al., 2009 [63]	Retrospective abattoir	Univariable analysis	Sheep and goats

Table S5: Associative studies of *E. granulosus* infection in intermediate hosts (cont.)

	study of 115,186 cattle and 99,401 sheep and goats in Arusha (Tanzania)		showed higher hydatid infection in 2005 (OR 2.2, $p<0.001$) and 2007 (OR 1.6, $p<0.001$) compared to cattle
Regassa et al., 2009 [42]	Abattoir survey of 415 cattle in Southern Ethiopia	Multivariable logistic regression	Host origin (OR 2.8, 95%CI 1.18, 6.51, $p=0.021$)
Kebede et al., 2009 [65]	Abattoir study of 420 cattle and 340 sheep in Bahir Dar (Ethiopia)	Univariable analysis	Host species ($p<0.001$)
Ibrahim, 2010 [47]	Abattoir survey of 2,668 cattle, 6,525 sheep, 3,578 goats and 140 camels in Al Baha (Saudi Arabia)	Multivariable logistic regression	Host species, age, gender (cattle and sheep) and seasonal variations (sheep and goats) ($p<0.05$)
Erbeto et al., 2010 [51]	Abattoir survey of 1,053 sheep and 639 goats in Addis Ababa (Ethiopia)	Univariable analysis	Host species, age, gender and type of production system ($p<0.05$)
Getaw et al., 2010 [66]	Abattoir survey of 852 cattle, 92 sheep and 208 goats in central Ethiopia	Univariable analysis	Host species ($p<0.001$)
Acosta-Jamett et al., 2010 [44]	Retrospective abattoir study of 174,034 cattle, 35,404 sheep, 22,208 goats, 25,355 pigs and 9,391 equines in Coquimbo (Chile)	Linear correlation (Spearman's rank coefficient)	Host species ($p<0.001$), geographic origin ($p<0.001$) and negative correlation between prevalence in goats and rainfall ($p=0.02$)
Zewdu et al., 2010 [59]	Abattoir survey of 384 zebu cattle in Ambo (Ethiopia)	Univariable analysis	Age ($p<0.0001$)
Bekele et al., 2011 [67]	Abattoir survey of 546 cattle in southern Ethiopia	Univariable analysis	Local breeds harboured higher infection levels ($p=0.043$)
Fromsa et al., 2011 [48]	Retrospective abattoir study of 22,863 cattle, 6,518 sheep, 1,753 goats, 417 camels and 150 pigs in Ethiopia	Univariable analysis	Host species ($p<0.001$), higher altitude (cattle) ($p<0.001$) and (sheep) ($p<0.01$)
Marshet et al., 2011 [57]	Abattoir survey of 611 sheep and 389 goats in Addis Ababa (Ethiopia)	Multivariable logistic regression	Host species (OR 5.14, 95%CI 2.76-9.55, $p<0.0001$) and age (OR 1.68, 95%CI 1.22-2.85, $p<0.029$)

Measures of association reported when available, except for Ibrahim (2010) due to the large number of odds ratios and corresponding confidence intervals calculated.
Abbreviations: OR, odds ratio; CI, confidence interval.

Table S6: Associative studies of *E. granulosus* infection in wild intermediate hosts

Table S6. Associative studies of *E. granulosus* infection in wild intermediate hosts

Reference	Study Information	Statistical Method	Significant Factor
McNeill et al., 1987 [71]	Parasitological examination of 580 lungs of moose (*Alces alces*) in southwestern Quebec (Canada)	Univariable analysis	Cyst intensity increased with moose age (p<0.01)
Messier et al., 1989 [70]	Parasitological examination of 232 lungs of moose (*Alces alces*) in southwestern Quebec (Canada)	Univariable analysis	Cyst prevalence and infection increased with moose density (p<0.01) and were correlated with moose age (p<0.01)
Barnes et al., 2007 [69]	Post mortem examination of 2,998 macropodids in Queensland (Australia)	Multivariable logistic regression	Eastern grey kangaroos females harbour more cysts than males (p=0.008)

Measures of association reported when available.

Table S7: Studies identifying significant determinants of infection of foxes with *E. multilocularis*

Table S7. Studies identifying significant determinants of infection of foxes with *E. multilocularis*

Reference	Study Information	Statistical Method	Significant Factor
Tackmann et al., 1998 [81]	Post mortem examination of 4,374 foxes in Brandenburg (Germany)	Univariable analysis	Under high endemic conditions juveniles were found more frequently infected than adults (p<0.001)
Morishima et al., 1999 [80]	Coproantigen detection and egg examination of 534 feacal samples of foxes in Hokkaido (Japan)	Univariable analysis	Juveniles (p<0.001) presented higher coproantigen positivity
Hofer et al., 2000 [76]	Post mortem examination of 388 red foxes in in Zurich (Switzerland)	Univariable analysis	Sub-adults (p<0.05) presented higher parasite burdens
Yimam et al., 2002 [77]	Post mortem examination of 67 red foxes in Otaru (Japan)	Univariable analysis	Juveniles (p<0.021) presented higher parasite burdens
Losson et al., 2003 [74]	Post mortem examination of 709 foxes in Wallonia (Belgium)	Univariable analysis	Juveniles (p=0.01) presented higher prevalence
Fischer et al., 2005 [78]	Post mortem examination of 267 foxes in Geneva (Switzerland)	Multivariable logistic regression	Juveniles (p=0.013) presented higher parasite burdens
Hegglin et al., 2007 [82]	Post mortem examination of 582 foxes in Zurich (Switzerland)	Multivariable logistic regression	Season * age (marked in juveniles) (AICc weight=0.69) and zone * age (marked for adults) (AICc weight=0.45) [1]
Brossard et al., 2007 [75]	Post mortem examination of 3,793 foxes in western Switzerland	Univariable analysis	Juveniles (p<0.001) presented higher prevalence and infection intensity
Ziadinov et al., 2010 [79]	Post mortem examination of 151 foxes in central Kyrgyzstan	Zero-inflated negative binomial model	Prevalence decreased with age (OR 1.28, 95%CI 1.01-1.62, p=0.042)
Bruzinskaitt-Schmidhalter et al., 2012 [83]	Post mortem examination of 310 red foxes in Lithuania	Multivariable logistic regression	Regressor parameters "juvenile" (95%CI [-0.54, -0.94]) and "male" (95%CI [1.20, 1.27]) were associated with parasite abundance

Measures of association reported when available
(*) Interaction term.
[1] The model explaining best the prevalence rate in foxes (lowest AICc) included the variables *Zone, season, age, zone × age, season × age*.
Abbreviations: OR, odds ratio; CI, confidence interval; AICc, Akaike's information criterion corrected for small samples sizes.

Table S8: Studies assessing association between *E. multilocularis* infection in foxes and environmental factors

Table S8. Studies assessing association between *E. multilocularis* infection in foxes and environmental factors

Reference	Study Information	Statistical Method	Significant Factor
Kritsky et al., 1978 [92]	Post mortem examination of 1,153 foxes in North Dakota (EE.UU)	Univariable analysis	Seasonal variation of prevalence (p=0.0131)
Tackmann et al., 1998 [81]	Post mortem examination of 4,374 foxes in Brandenburg (Germany)	Univariable analysis	Variations in prevalence among 3 geographic zones (p<0.001)
Hofer et al., 2000 [76]	Post mortem examination of 388 red foxes in in Zurich (Switzerland)	Univariable analysis	Seasonal variation in prevalence in urban sub-adult males (p<0.001)
Raoul et al. 2001 [88]	Post mortem examination of 222 red foxes in in Franche-Comté (France)	Univariable analysis	Higher prevalence found in mid-altitude areas compared to low altitude areas (p<0.001)
Denzin et al., 2005 [91]	Post mortem examination of 1,341 red foxes in Saxony-Anhalt	Multivariable logistic regression	Negative association with probability of infestation and the average annual maximum temperature (p=0.00001)
König et al. 2005 [84]	Post mortem examination of 268 foxes in Bavaria (Germany)	Univariable analysis	Variations in prevalence among 3 geographic areas (p<0.001)
Miterpáková et al., 2006 [89]	Parasitological examination of 3,096 foxes in Slovakia	Simple correlation	Prevalence (p=0.021) and abundance (p=0.020) correlated with mean annual precipitation
Dubinsky et al., 2006 [85]	Parasitological examination of 392 foxes in Poland	Univariable analysis	Higher prevalence in the Polish border area with Slovakia (p=0.0009)
Hegglin et al., 2007 [82]	Post mortem examination of 582 foxes in Zurich (Switzerland)	Multivariable logistic regression	Season (AICc weight=1) (i.e. summer/autumn vs winter, OR 0.78, 95%CI 0.38-1.61) and season * age (marked in juveniles) (AICc weight=0.69) [1]
Brossard et al., 2007	Post mortem	Univariable analysis	Variations in

Table S8: Studies assessing association between *E. multilocularis* infection in foxes and environmental factors (cont.)

[75]	examination of 3,793 foxes in western Switzerland		prevalence among geographic areas and seasons depending on host age (p<0.05)
Hanosset et al., 2008 [93]	Post-mortem examination of 990 foxes in Wallonia (Belgium)	Univariable analysis	Seasonal variations in prevalence. Summer/autumn, vs. winter/spring (OR 1.4, 95%CI 1.04–1.98, p=0.03)
Immelt et al., 2009 [87]	Post mortem examination of 959 foxes in South Hesse and Middle Hesse (Germany)	Multivariable logistic regression	Higher parasite burdens associated with areas with high agriculture land and high amount of precipitation (p<0.0001)
Miterpáková et al., 2009 [90]	Post mortem examination of 4,026 foxes in the Slovak Republic	Simple correlation and multivariable logistic regression	Correlation between the mean annual precipitation and both prevalence (p=0.022) and worm burden (p=0.021). Regional differences in prevalence (p<0.001)
Casulli et al., 2010 [86]	Post-mortem examination of 840 foxes in Hungary	Univariable analysis	Prevalence and abundance higher in the north-western half than in the south-eastern half of the country (p<0.001)

Measures of association reported when available
(*) Interaction term.
[1] The model explaining best the prevalence rate in foxes (lowest AICc) included the variables *Zone, season, age, zone × age, season × age*.
Abbreviations: OR, odds ratio; CI, confidence interval; AICc, Akaike's information criterion corrected for small samples sizes.

Table S9: Spatial studies of *E. multilocularis* in foxes

Table S9. Spatial studies of *E. multilocularis* in foxes

Reference	Study Information	Statistical Method	Significant Factor
Berke, 2001 [94]	Choropleth mapping of regional prevalence estimates based on parasitological examination from 5,365 foxes in Lower Saxony (Germany)	Conditional autoregressive spatial modelling	Raised prevalence in the southern and northern parts modeled with a second-order polynomial model ($p \leq 0.05$)
Staubach et al., 2001 [98]	Spatial analysis of infection status of 3,521 foxes on the background of geographic vector data in Brandenburg (Germany)	Univariable analysis	Infected foxes were collected closer to water bodies ($p=0.0048$), areas of high soil humidity ($p=0.013$) and on pastures ($p=0.078$)
Berke et al., 2002 [95]	Spatial analysis of parasitological examination of 5,365 red foxes in Lower Saxony (Germany)	Spatial scan statistic	Identification of disease cluster area from 1991-1997 (RR 4.80, 95%CI 4.11-5.63, $p=0.001$)
Pleydell et al., 2004 [97]	Spatial investigation of coproantigens patterns of 345 faecal samples from foxes in the Franche-Comté region (France)	Non-linear regression and semivariogram	The inclusion of the grassland index improved consistently the fitting of the models ($p<0.05$)
Denzin et al., 2005 [91]	Post mortem examination of 1,341 red foxes in Saxony-Anhalt	Spatial scan statistic	Identification of a clusters with increased risk of infection (RR 4.4, 95%CI 2.6-5.0, $p=0,001$)
Berke et al., 2008 [96]	Spatial-temporal analyses of parasitological examination of 8,459 foxes in Lower Saxony (Germany)	Spatial scan statistic	Fox infection was clustered in the southern part ($p \leq 0.01$)
Fuglei et al., 2008 [99]	Spatial coproantigen investigation of 473 artic fox faecal samples from Svalbard (Norway)	Estimation of fox feces densities by line transect methods and score confidence limits for the proportions	Highest proportion of fox coproantigen positive feces overlapped voles' geographical range ($p \leq 0.05$)

Measures of association reported when available
Abbreviations: RR, risk ratio; OR, odds ratio; CI, confidence interval; AIC, Akaike information criterion.

Table S10: Studies assessing association between *E. multilocularis* infection in foxes and host population factors

Table S10. Studies assessing association between *E. multilocularis* infection in foxes and host population factors

Reference	Study Information	Statistical Method	Significant Factor
Saitoh et al., 1998 [103]	Post mortem examination of 9,828 red foxes in Hokkaido (Japan)	Multivariable logistic regression	Vole abundance affected infection rates in foxes (p<0.001)
Hofer et al., 2000 [76]	Post mortem examination of 388 red foxes in in Zurich (Switzerland)	Univariable analysis	Higher prevalence in foxes from rural areas (p<0.01) vs. urban areas during winter
Stieger et al., 2002 [102]	Coproantigen examination of 604 fox faecal samples in Zurich (Switzerland)	Univariable analysis	Higher positive results in border and peri-urban zone compare to urban zone during winter (p<0.01)
Raoul et al., 2003 [105]	Coproantigen examination of 156 fox faecal samples in Le Souillot (France)	Univariable analysis	Decrease of infection as fox numbers reduce (p=0.0004)
Fischer et al., 2005 [78]	Post mortem examination of 267 foxes in Geneva (Switzerland)	Multivariable logistic regression	Level of urbanization. Rural vs urban (OR 2.73, 95%CI 1.24-5.97, p=0.012) and border vs urban (OR 2.32, 95%CI 1.03-5.18, p=0.04)
Tanner et al., 2006 [104]	Post mortem examination of 543 foxes in Grisons (Switzerland)	Linear correlation (Spearman's rank coefficient)	Prevalence correlated with predation on intermediate hosts (*Microtus/Pitymys*) (p=0.018)
Miterpáková et al., 2006 [89]	Parasitological examination of 3,096 foxes in Slovakia	Simple correlation	Prevalence correlated with density of small mammals (p=0.022)
Reperant et al., 2007 [100]	Post mortem examination of 228 red foxes in Geneva (Switzerland)	Multivariable logistic regression	Decrease prevalence from rural to urban areas (p=0.037)
Hegglin et al., 2007 [82]	Post mortem examination of 582 foxes in Zurich (Switzerland)	Multivariable logistic regression	Type of urbanization zone (AICc weight=1) (i.e. Border vs. peri-urban, OR 0.46 95%CI 0.25-0.85) and zone * age (marked for adults) (AICc weight=0.45) [1]
Hanosset et al., 2008	Post mortem	Linear correlation	Positive correlation

Table S10: Studies assessing association between *E. multilocularis* infection in foxes and host population factors (cont.)

[93]	examination of 990 foxes in Wallonia (Belgium)	(Spearman's rank coefficient)	between prevalence in foxes and muskrats (Spearman's rank correlation coefficient=1, p<0.0001)
Robardet et al., 2008 [101]	Post mortem examination of 127 red foxes in Nancy (France)	Multivariable logistic regression	Type of urbanization zone (AICc weight=0.94). Urban vs. rural (OR 0.04, 95%CI 0.01-0.14) and peri- urban vs. rural (OR 0.38, 95%CI 0.14-1.01).

Measures of association reported when available
(*) Interaction term.
[1] The model explaining best the prevalence rate in foxes (lowest AICc) included the variables *Zone, season, age, zone × age, season × age*.
Abbreviations: OR, odds ratio; CI, confidence interval; AICc, Akaike's information criterion corrected for small samples sizes.

Table S11: Associative studies of *E. multilocularis* infection in carnivores, other than foxes

Table S11. Associative studies of *E. multilocularis* infection in carnivores, other than foxes

Reference	Study Information	Statistical Method	Significant Factor
Budke et al., 2005 [12]	Arecoline purgation of 371 owned dogs in the Tibetan Plateau (China)	Multivariable logistic regression	Dogs being allowed to roam were more likely of be infected (OR 0.3693, 95%CI 0.1593–0.8558, *p*=0.02)
Wang et al., 2007 [106]	Collection of faecal samples and arecoline purgation of 252 dogs in the Shiqu County (China)	Multivariable conditional logistic regression	Canine infection related with the density of small mammal burrows in the open pastures (OR=1.048, *p*=0.003)
Ziadinov et al., 2008 [109]	Arecoline purgation of 466 owned dogs in At-Bashy (Kyrgyzstan)	Multivariable logistic regression	Dogs being allowed to roam (OR 0.39, 95%CI 0.199– 0.749, *p*=0.0056) and hunting dogs (OR 4.2, 95%CI 1.89–9.68, *p*=0.0005) were more likely of be infected
Dyachenko et al., 2008 [110]	Cross-sectional survey of faecal samples of 17,894 dogs and 9,064 cats in Germany	Univariable analysis	Higher dog prevalence found in the south compared with the north (OR 2.6, 95%CI 1.4-4.9, *p*<0.01)
Antolova et al., 2009 [108]	Coprological examination of 289 dogs in Slovakia	Multivariable logistic regression	Dogs being fed with raw offal (OR 7.05, 95%CI 1.24-40.09, *p*=0.025) and dogs that used to catch rodents (OR 6.09, 95%CI 1.16-32.01, *p*=0.04) were more likely to be infected
Wang et al., 2010 [107]	Arecoline purgation of 228 owned dogs Shiqu County (China)	Multivariable logistic regression	Parasite burden in dogs was related to the maximum burrow density of intermediate host *Ochotona spp.* (*p*=0.022)
Liccioli et el., 2012 [111]	Post-mortem examination of 61 coyotes in Calgary (Canada)	Univariable analysis	Higher prevalence in juveniles (*p*=0.035)
Catalano et al., 2012 [112]	Post-mortem examination of 91 coyotes in Alberta (Canada)	Univariable analysis	Higher infection in male than female coyotes (*p*=0.05)
Bruzinskaite-Schmidhalter et al., 2012 [83]	Post mortem examination of 310 red foxes in Lithuania	Multivariable logistic regression	Higher parasite abundance in raccoon dogs in autumn than winter (RR 0.002, 95%CI 0.0005-0.01)

Measures of association reported when available
Abbreviations: OR, odds ratio; RR, risk ratio; CI, confidence interval

Table S12: Associative studies on *E. multilocularis* infection in intermediate hosts

Table S12. Associative studies on *E. multilocularis* infection in intermediate hosts

Reference	Study Information	Statistical Method	Significant Factor
Leiby et al., 1974 [115]	Parasitological examination of 5,638 *Peromyscus maniculatus* in North Dakota (EEUU)	Analysis of variance	Age (p=0.0001), habitat (p=0.0002), season (p=0.002), age*season (p=0.003) and habitat*season (p=0.04)
Gottstein et al., 2001 [114]	Parasitological examination of 513 rodents in Fribourg (Switzerland)	Univariable analysis	Yearly fluctuation of prevalence (p<0.005) for *Arvicola terrestris*
Henttonen et al., 2001 [119]	Parasitological examination of 224 *Microtus rossiaemeridionalis* in Svalbard (Norway)	Multivariable logistic regression	Overwintered adults (p<0.001) and prevalence variation related with body weight and length (p<0.001)
Stieger et al., 2002 [102]	Parasitological examination of 1,155 rodents in Zurich (Switzerland)	Univariable analysis	Adults (p<0.001) showed higher prevalence and prevalence variation by trapping site (p=0.019) for *A. terrestris*
Hanosset et al., 2008 [93]	Parasitological examination of 1,249 rodents in Wallonia (Belgium)	Univariable analysis	Adult muskrats *Ondatra zibethicus* (p=6.56x10^{-6}) presented higher prevalence
Reperant et al., 2009 [117]	Parasitological examination of 658 rodents in Geneva (Switzerland)	Multivariable logistic regression	Body weight and geographical area (p<0.0001) for *Arvicola terrestris*
Stien et al., 2010 [118]	Parasitological examination of 387 sibling voles in Svalbard (Norway)	Multivariable logistic regression	Sample site and vole length (p<0.0001), year of sampling, sample site * year and sample site vole length (p=0.02)
Burlet et al., 2011 [116]	Parasitological examination of 856 *A. terrestris* in Zurich (Switzerland)	Multivariable logistic regression	Age (>7 months), period, area and mean day temperature included in the best-fitting model with the lowest AICc (-244.04)

Measures of association reported when available
(*) Interaction term.

Abbreviations: OR, odds ratio; CI, confidence interval; AICc, Akaike's information criterion corrected for small samples sizes.

Appendix: Supporting information-Paper 2

Text S1: Estimating the marginal likelihood

Supporting Information Text S1

Estimating the Marginal Likelihood

The marginal likelihood was estimated using the standard Laplace analytical approximation. Numerically, this was somewhat involved in order to ensure reliable estimates due to the need to use finite differencing rather than analytical derivatives. The computational code was written in C and called from within R using the R API. Extensive use was made of both the GNU Scientific Library (http://www.gnu.org/software/gsl/) and also some of R's internal optimization functions. The likelihood $\times$ prior function was optimized using R's L-BFGS-B (called internally from C), where at each step in the optimization the given system of ordinary differential equations (ODE) in the likelihood function was solved numerically, using GSL's ODE solver functionality with adaptive step-size and error controlling routines (in particular the explicit embedded Runge-Kutta Prince-Dormand (8, 9) method was used). The gradient function in the L-BFGS-B was provided via using GSL's adaptive finite difference routines. The Hessian estimate in the Laplace approximation was computed using finite differencing (again using GSL's routines) but where this was first nested inside a one dimensional minimiser in order to determine the initial step size value (provided to the GSL routines) in the finite difference approximation which resulted in the smallest (absolute) error between the estimate of the Hessian using a five point difference rule and a three point difference rule. The step size optimization was performed using GSL's Brent minimization algorithm. Once the optimal step size had been determined then the final, most robust value of the Hessian was determined. This rather lengthy approach was used as Hessian estimates using finite differencing can be rather sensitive to the step size used (or in this case the initial step size guess suggested to the adaptive GSL routines). This general approach is what is used in the R abn library and has been tested for robustness on a number of data sets.

Text S2: Results using an uninformative prior for μ

Supporting Information Text S2

Results using an uniformative prior for μ

This is similar to Table 1 in the main manuscript but for the uninformative prior for μ.

Model	Description	Prior for μ	Log marginal likelihood
1-C	no immunity ($\alpha = 0$) Constant FOI: $\log \beta(a) = \beta_0$	$N(0.0, \sqrt{1000})$	-303.2 ($\Delta_{ML} = 16.4$)
1-L	no immunity ($\alpha = 0$) Linear FOI: $\log \beta(a) = \beta_0 + \beta_1 a$	$N(0.0, \sqrt{1000})$	-306.4 ($\Delta_{ML} = 22.8$)
1-Q	no immunity ($\alpha = 0$) Quadratic FOI: $\log \beta(a) = \beta_0 + \beta_1 a + \beta_2 a^2$	$N(0.0, \sqrt{1000})$	-300.3 ($\Delta_{ML} = 10.6$)
1-P	no immunity ($\alpha = 0$) Periodic FOI: $\log\{\beta(a)\} = \beta_0 + \beta_1 \sin\left\{2\pi\left(a - \frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(0.0, \sqrt{1000})$	-295.0 ($\Delta_{ML} = 0.0$)
2	lifelong immunity ($\gamma = 0$) periodic FOI: $\log\{\beta(a)\} = \beta_0 + \beta_1 \sin\left\{2\pi\left(a - \frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(0.0, \sqrt{1000})$	-296.2 ($\Delta_{ML} = 2.4$)
3	transient immunity ($\gamma \neq 0$) periodic FOI: $\log\{\beta(a)\} = \beta_0 + \beta_1 \sin\left\{2\pi\left(a - \frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(0.0, \sqrt{1000})$	-297.4 ($\Delta_{ML} = 4.8$)

Text S3: Modelling results for foxes of all ages

Supporting Information Text S3

Modeling results for foxes of all ages

This is similar to Table 1 in the main manuscript but for the models fitting to the data from foxes of all ages.

Model	Description	Prior for μ	Log marginal likelihood
1-C	no immunity ($\alpha = 0$) Constant FOI: $\log \beta(a) = \beta_0$	$N(1.2, 0.2)$ $N(1.3, 0.3)$	-354.6 ($\Delta_{ML} = 22.8$) -354.0 ($\Delta_{ML} = 21.6$)
1-L	no immunity ($\alpha = 0$) Linear FOI: $\log \beta(a) = \beta_0 + \beta_1 a$	$N(1.2, 0.2)$ $N(1.3, 0.3)$	-360.6 ($\Delta_{ML} = 34.8$) -359.6 ($\Delta_{ML} = 32.8$)
1-Q	no immunity ($\alpha = 0$) Quadratic FOI: $\log \beta(a) = \beta_0 + \beta_1 a + \beta_2 a^2$	$N(1.2, 0.2)$ $N(1.3, 0.3)$	-365.5 ($\Delta_{ML} = 44.6$) -365.1 ($\Delta_{ML} = 43.8$)
1-P	no immunity ($\alpha = 0$) Periodic FOI: $\log\{\beta(a)\} = \beta_0 + \beta_1 \sin\left\{2\pi\left(a - \frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(1.2, 0.2)$ $N(1.3, 0.3)$	-343.3 ($\Delta_{ML} = 0.2$) -343.2 ($\Delta_{ML} = 0.0$)
2	lifelong immunity ($\gamma = 0$) periodic FOI: $\log\{\beta(a)\} = \beta_0 + \beta_1 \sin\left\{2\pi\left(a - \frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(1.2, 0.2)$ $N(1.3, 0.3)$	-344.6 ($\Delta_{ML} = 2.8$) -344.8 ($\Delta_{ML} = 3.2$)
3	transient immunity ($\gamma \neq 0$) periodic FOI: $\log\{\beta(a)\} = \beta_0 + \beta_1 \sin\left\{2\pi\left(a - \frac{\exp(a_s)}{1+\exp(a_s)}\right)\right\}$	$N(1.2, 0.2)$ $N(1.3, 0.3)$	-345.7 ($\Delta_{ML} = 5.0$) -349.3 ($\Delta_{ML} = 12.2$)

Text S4: Estimates of the posterior modes for all the parameters in models presented in Table 1

Supporting Information Text S4

Estimates of the posterior modes for all the parameters in models presented in Table 1

The following table gives estimates for the parameter modes in each of the models presented in Table 1 in the main text. NA - not applicable.

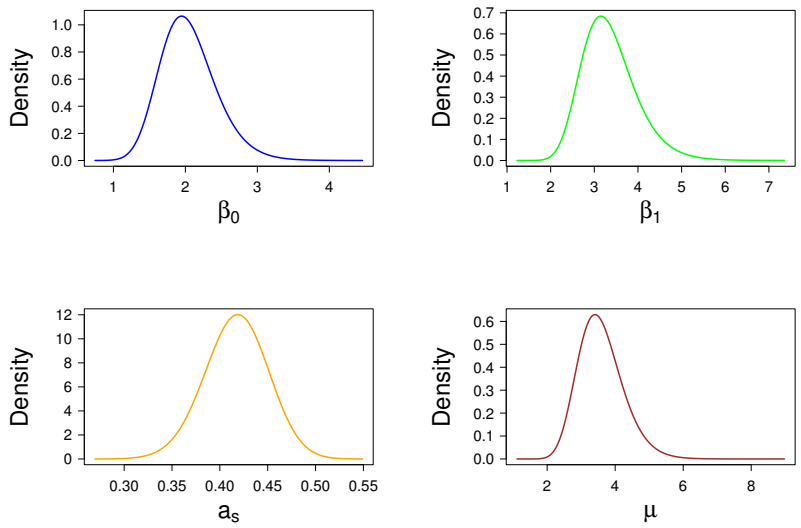
Model	Prior for μ	Posterior modes
1-C	$N(1.2, 0.2)$	$\{\alpha, \beta_0, \mu, \gamma\} = \{0, 0.64, 0.79, \text{NA}\}$
	$N(1.3, 0.3)$	$\{\alpha, \beta_0, \mu, \gamma\} = \{0, 0.50, 0.57, \text{NA}\}$
1-L	$N(1.2, 0.2)$	$\{\alpha, \beta_0, \beta_1, \mu, \gamma\} = \{0, 0.55, 0.23, 0.92, \text{NA}\}$
	$N(1.3, 0.3)$	$\{\alpha, \beta_0, \beta_1, \mu, \gamma\} = \{0, 0.46, 0.18, 0.74, \text{NA}\}$
1-Q	$N(1.2, 0.2)$	$\{\alpha, \beta_0, \beta_1, \beta_2, \mu, \gamma\} = \{0, -0.02, 2.10, -0.70, 1.12, \text{NA}\}$
	$N(1.3, 0.3)$	$\{\alpha, \beta_0, \beta_1, \beta_2, \mu, \gamma\} = \{0, -0.02, 2.09, -0.70, 1.12, \text{NA}\}$
1-P	$N(1.2, 0.2)$	$\{\alpha, \beta_0, \beta_1, a_s, \mu, \gamma\} = \{0, 0.72, 1.19, -0.32, 1.28, \text{NA}\}$
	$N(1.3, 0.3)$	$\{\alpha, \beta_0, \beta_1, a_s, \mu, \gamma\} = \{0, 0.83, 1.19, -0.29, 1.42, \text{NA}\}$
2	$N(1.2, 0.2)$	$\{\alpha, \beta_0, \beta_1, a_s, \mu, \gamma\} = \{-5.30, 0.76, 1.19, -0.30, 1.30, 0\}$
	$N(1.3, 0.3)$	$\{\alpha, \beta_0, \beta_1, a_s, \mu, \gamma\} = \{-4.83, 0.83, 1.20, -0.27, 1.39, 0\}$
3	$N(1.2, 0.2)$	$\{\alpha, \beta_0, \beta_1, a_s, \mu, \gamma\} = \{-4.56, 0.72, 1.18, -0.32, 1.28, 2.72\}$
	$N(1.3, 0.3)$	$\{\alpha, \beta_0, \beta_1, a_s, \mu, \gamma\} = \{-6.32, 0.83, 1.19, -0.28, 1.43, 2.76\}$

Text S5: Full marginal posterior densities for model 1-P for the parameters β_0, β_1, a_s and μ using the informative prior μ with mean = 1.2 and s.d. =0.2

Supporting Information Text S5

Full marginal posterior densities for model 1-P

This section provides marginal posterior density estimates for all parameters in Model 1-P.

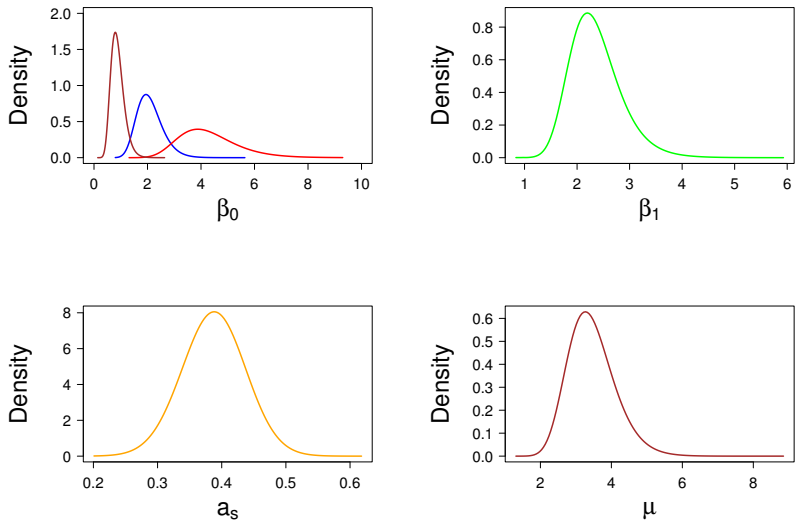


Marginal posterior densities for β_0, β_1, a_s and μ on the real scale using the informative prior for μ with mean=1.2 and sd=0.2

Text S6: Full marginal posterior densities for model 1-P_0 for the parameters β_0, β_1, a_s and μ using the informative prior μ with mean =1.2 and s.d.= 0.2

Supporting Information Text S6

Full marginal Posterior densities for model 1-P_0

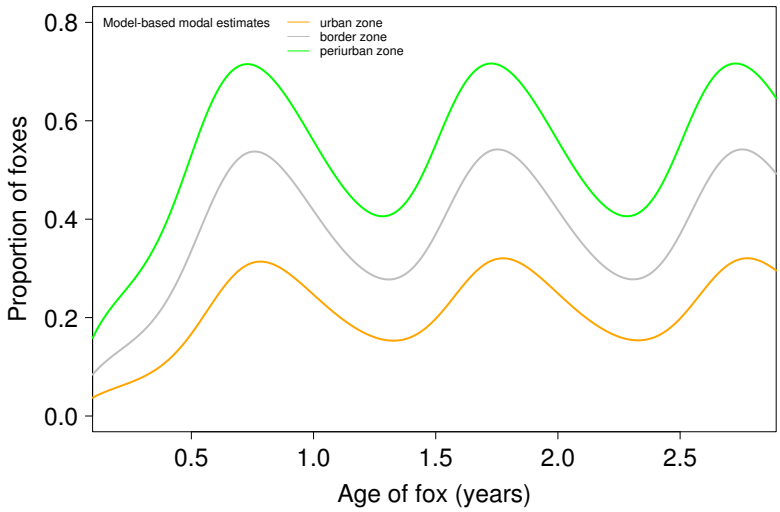


Marginal posterior densities for β_0, β_1, a_s and μ on the real scale using the informative prior for μ with mean=1.2 and sd=0.2. For β_0 brown is urban, blue is border and red is periurban.

Text S7: Model prevalence estimates by habitat using model 1-P_0

Supporting Information Text S7

Modal prevalence estimates by habitat using Model 1-P_0



Model predicted prevalence using posterior mode estimates of all model parameters and using the informative prior for μ with mean=1.2 and sd=0.2.

Appendix: Supporting information-Paper 3

Text S2: R code

Additional file 2. R Code

1. Estimating parameters

```
library(deSolve)
library(MASS)

#load the data
abunP<-read.csv("abundP.csv", header=T)) #periurban data
abunB<-read.csv("abunB.csv", header=T)) #border data
abunU<-read.csv("abunU.csv", header=T) #urban data


# periurban zone- differential equation for model 20
ech1<-function(t, state, parms) {
  with(as.list(c(state, parms)), {
    dM <- exp(-x*t)*exp(b0-b*sin(2*pi*t))-8.625*M
    return(list(c( dM)))
  })
}
state <- c(M=0)

# border zone - differential equation for model 20
ech2<-function(t,state,parms){
  with(as.list(c(state,parms)),{
    dMbor<-exp(b0-bbor*sin(2*pi*t))-8.625*Mbor
    return(list(c(dMbor)))
  })
}

statebor <- c(Mbor=0)

# urban zone- differential equation for model 20
ech3<-function(t, state, parms) {
  with(as.list(c(state, parms)), {
    dMurb <- exp(b0-burb*sin(2*pi*t))-8.625*Murb
    return(list(c( dMurb)))
  })
}
stateurb <- c(Murb=0)


#inserts a first row of 0 counts at time=0 to allow initiating numerical integration.
initb=data.frame(Age_days=0, Zone="border", Abun=0, years=0)
abunB<-rbind(initb, abunB)
initp=data.frame(Age_days=0, Zone="periurban", Abun=0, years=0)
abunP<-rbind(initp, abunP)
initu=data.frame(Age_days=0, Zone="urban", Abun=0, years=0)
abunU<-rbind(initu, abunU)


#likelihood function
pred20 <- function(parms) {
  out1 <- ode(y = state, func = ech1, parms = parms, times = abunP$years)
  neglogkp<- -sum(dnbinom(x=abunP$Abun,size= 0.09758278, mu=out1[,"M"], log = TRUE)) #
periurban
out2 <- ode(y = statebor, func = ech2, parms = parms, times = abunB$years)
  neglogkb<- -sum(dnbinom(x=abunB$Abun, size=0.05246528, mu=out2[,"Mbor"], log = TRUE)) #
border
  out3 <- ode(y = stateurb, func = ech3, parms = parms, times = abunU$years)
```

Text S2: R code (cont.)

```
 neglogku<- -sum(dnbinom(x=abunU$Abun, size=0.01560734, mu=out3[,"Murb"], log = TRUE)) #
urban
   if(is.infinite(neglogkp)){return(1e7)}
 if(is.infinite(neglogkb)){return(1e7)}
 if(is.infinite(neglogku)){return(1e7)}
 tot=neglogkp+neglogkb+neglogku
 return(tot)
}


#loading starting parameters
start<-c(x=0.5, b0=8,b=2,burb=2, bbor=2)

#Minimizing the negative log likelihood
fit1<- optim(par=start,
             fn=pred20, control=c(maxit=10000))

# periurban immunity – alternative differential equation for model 19 to substitute in likelihood
function.
ech1<-function(t, state, parms) {
 with(as.list(c(state, parms)), {
   dS  <- g*(1-S)-a*(exp(-x*t)*exp(b0-b*sin(2*pi*t)))*S
   dM <- (exp(-x*t)*exp(b0-b*sin(2*pi*t)))*S - muu*M
   return(list(c(dS,dM)))
 })
}

state <- c(S=1, M=0)
start<-c(x=0.5,b0=8, b=2.6, burb=2, bbor=2,a=1, g=8.2,muu=4)
```

2. Bootstrap Cis (model 17)

```
library(deSolve)
library(MASS)

# periurban zone- differential equation for model 17
ech1<-function(t, state, parms) {
 with(as.list(c(state, parms)), {
   dM <- exp(-x*t)*exp(b0-b*sin(2*pi*t))-8.625*M
   return(list(c( dM)))
 })
}
state <- c(M=0)

# border zone - differential equation for model 17
ech2<-function(t,state,parms){
 with(as.list(c(state,parms)),{
   dMbor<-exp(b0-bbor*sin(2*pi*t))-8.625*Mbor
   return(list(c(dMbor)))
 })
}

statebor <- c(Mbor=0)

# urban zone- differential equation for model 17
ech3<-function(t, state, parms) {
 with(as.list(c(state, parms)), {
   dMurb <- exp(b0-burb*sin(2*pi*t))-8.625*Murb
   return(list(c( dMurb)))
```

Text S2: R code (cont.)

```
  })
}
stateurb <- c(Murb=0)

#state initial bounds
start=c(b=2.56,b0=8.45 ,  x=0.53,burb=1.20,bbor=0.13)

#Minimizing the negative log likelihood
fit1<- optim(par=start,
          fn=pred20, control=c(maxit=10000))

#Save results
newres<-as.data.frame(fit1$par)
mle1<-as.data.frame(fit1$value)


for(i in 1:1000) {
#bootstrap new data set for periurban zone
newP<-abunP[sample(1:nrow(abunP), 185, replace=TRUE),]
newP1<-newP[order(newP$years),]
initp=data.frame(Age_days=0, Zone="periurban", Abun=0, years=0)
newP1<-rbind(initp, newP1)
pk<-glm.nb(newP$Abun~1)$theta

 #bootstrap new data set for Border zone
newB<-abunB[sample(1:nrow(abunB), 200, replace=TRUE),]
newB1<-newB[order(newB$years),]
initb=data.frame(Age_days=0, Zone="border", Abun=0, years=0)
newB1<-rbind(initb, newB1)
bk<-glm.nb(newB$Abun~1)$theta
#bootstrap new data set for urban zone
newU<-abun U[sample(1:nrow(abunU), 146, replace=TRUE),]
newU1<-newU[order(newU$years),]
initu=data.frame(Age_days=0, Zone="urban", Abun=0, years=0)
newU1<-rbind(initu, newU1)
uk<-glm.nb(newU$Abun~1)$theta
}


#three populations - calculating the likelihood - predicted abundance given parameters
  pred10 <- function(parms) {
    out1 <- ode(y = state, func = ech1, parms = parms, times = newP1$years)
    neglogkp<- -sum(dnbinom(x=newP1$Abun,size=pk, mu=out1[,"M"], log = TRUE))  #periurban
    out2 <- ode(y = statebor, func = ech2, parms = parms, times = newB1$years)
    neglogkb<- -sum(dnbinom(x=newB1$Abun,size=bk, mu=out2[,"Mbor"], log = TRUE))  #border
    out3 <- ode(y = stateurb, func = ech3, parms = parms, times = newU1$years)
    neglogku<- -sum(dnbinom(x=newU1$Abun,size=uk, mu=out3[,"Murb"], log = TRUE)) # urban
    if(is.infinite(neglogkp)){return(1e7)}
    if(is.infinite(neglogkb)){return(1e7)}
    if(is.infinite(neglogku)){return(1e7)}
    tot=neglogkp+neglogkb+neglogku
    return(tot)
  }

#Minimizing the negative log likelihood
fit10<- optim(par=fit10$par,
                    fn=pred10, control=c(maxit=10000))

#Save results
newres<-cbind(newres, as.data.frame(fit1$par)) #appends the results of each iteration
```

Text S2: R code (cont.)

```
mle1<-cbind(mle1, as.data.frame(fit1$value)) #appends the results of NegLogLkh each iteration
```

3. Estimating Confidence bands and plotting

```
library(matrixStats)
times1<-seq(0.15, 4, by=0.01) #for plots from 2 months of age

#periurban
hres<- function (t){
  h<-exp(-x*t)*exp(b0-b*sin(2*pi*t))
  return (h)
}

b0=newres[2,1]
b=newres[3,1]
burb=newres[4,1]
bbor=newres[5,1]
x=newres[1,1]

rest<-as.data.frame(hres(times1))
rest1<-rest


for (i in 2:1000){
  b0=newres[2,i]
  b=newres[3,i]
  x=newres[1,i]
  rest<-as.data.frame(hres(times1))
  rest1<-cbind(rest1, rest)
}

bandsP<-rowQuantiles(as.matrix(rest1), probs=c(0.025,0.5,0.975))

#border
hres2<-function (t){
  hb<-exp(b0-bbor*sin(2*pi*t))
  return(hb)
}

b0=newres[2,1]
bbor=newres[5,1]
rest<-as.data.frame(hres2(times1))
restB<-rest

for (i in 2:1000){
  b0=newres[2,i]
  bbor=newres[5,i]
  rest<-as.data.frame(hres2(times1))
  restB<-cbind(restB, rest)
}
bandsB<-rowQuantiles(as.matrix(restB), probs=c(0.025,0.5,0.975))

#urban
hres3<-function (t){
  hu<-exp(b0-burb*sin(2*pi*t))
  return(hu)
}

b0=newres[2,1]
burb=newres[4,1]
```

Text S2: R code (cont.)

```
rest<-as.data.frame(hres3(times1))
restU<-rest

for (i in 2:1000){
  b0=newres[2,i]
  burb=newres[4,i]
  rest<-as.data.frame(hres3(times1))
  restU<-cbind(restU, rest)
}

bandsU<-rowQuantiles(as.matrix(restU), probs=c(0.025,0.5,0.975))


##Infection pressure 3 zones
tiff(file="allzones.tiff", compression="lzw",res=600, width=10, height=3, units="in")

par(mfrow=c(3,1),mar=c(8,8,2,4))

#periurban
lablist.y<-as.vector(c("0", "3e4", "6e4", "9e4"))
lablist.x<-as.vector(c("0", "1", "2", "3", "4"))
plot(times1, bandsP[,2], ylim=c(0, 90000), type="l", lwd=2, axes=FALSE, xlab="", ylab="")
axis(2, at=seq(0,90000, by =30000), labels=FALSE)
axis(1, at=seq(0,4, by=1), labels=FALSE)
text(y=seq(0, 90000, by=30000), par("usr")[1],labels=lablist.y, pos=2, offset=1, xpd=TRUE, cex=0.6)
text(x=seq(0, 4, by=1), par("usr")[1],labels=lablist.x, pos=1, offset=1, xpd=TRUE, cex=0.6)
title(xlab="Age(years)", line=1.5, cex.lab=0.8)
title (ylab="Infection Pressure", line=2, cex.lab=0.8)
lines(times1,bandsh[,1],col="red",lwd=1)
lines(times1,bandsh[,3],col="red",lwd=1)
mtext("Periurban Foxes",  cex=0.8, font=1,line=-8, side=1.5)

#border
lablist.y<-as.vector(c("0", "3e4", "6e4", "9e4"))
lablist.x<-as.vector(c("0", "1", "2", "3", "4"))
plot(times1, bandsB[,2], ylim=c(0, 90000), type="l", lwd=2, axes=FALSE, xlab="", ylab="")
axis(2, at=seq(0,90000, by =30000), labels=FALSE)
axis(1, at=seq(0,4, by=1), labels=FALSE)
text(y=seq(0, 90000, by=30000), par("usr")[1],labels=lablist.y, pos=2, offset=1, xpd=TRUE, cex=0.6)
text(x=seq(0, 4, by=1), par("usr")[1],labels=lablist.x, pos=1, offset=1, xpd=TRUE, cex=0.6)
title(xlab="Age(years)", line=1.5, cex.lab=0.8)
title (ylab="Infection Pressure", line=2, cex.lab=0.8)
lines(times1,bandsB[,1],col="red",lwd=1)
lines(times1,bandsB[,3],col="red",lwd=1)
mtext("Border Foxes", cex=0.8, font=1,line=-8, side=1.5)

#urban

lablist.y<-as.vector(c("0", "3e4", "6e4", "9e4"))
lablist.x<-as.vector(c("0", "1", "2", "3", "4"))
plot(times1, bandsU[,2], ylim=c(0, 90000), type="l", lwd=2, axes=FALSE, xlab="", ylab="")
axis(2, at=seq(0,90000, by =30000), labels=FALSE)
axis(1, at=seq(0,4, by=1), labels=FALSE)
text(y=seq(0, 90000, by=30000), par("usr")[1],labels=lablist.y, pos=2, offset=1, xpd=TRUE, cex=0.6)
text(x=seq(0, 4, by=1), par("usr")[1],labels=lablist.x, pos=1, offset=1, xpd=TRUE, cex=0.6)
title(xlab="Age(years)", line=1.5, cex.lab=0.8)
title (ylab="Infection Pressure", line=2, cex.lab=0.8)
lines(times1,bandsU[,1],col="red",lwd=1)
lines(times1,bandsU[,3],col="red",lwd=1)
mtext("Urban Foxes", cex=0.8, font=1,line=-8, side=1.5)



  dev.off()
```

Appendix: Supporting information-Paper 4

Text S2: Bayesian latent class model code for three diagnostic tests

Additional file 2. Bayesian latent-class model code for three diagnostic tests

```
############################################################
##Definition of the variables in the model
############################################################

var p[N], q[N,8], pr[N], L[N],checks[N,16];

#N <- observations (N=300 foxes)
# p <- individual samples
# q <- different combinations of test results
# pr <- prevalence
# s <- test sensitivities
# c <- test specificities
#  cs <- conditional dependency between tests sensitivities
#  cc <- conditional dependency between tests specificities
# m.short <- data set name


############################################################
## Modelling the different probabilities of combinations of tests results
############################################################

model {

 for(i in 1:N){

q[i,1]<-pr[i]*(s1*s2*s3+cs12+cs13+cs23)+(1-pr[i])*((1-c1)*(1-c2)*(1-
c3)+cc12+cc13+cc23);
   q[i,2]<-pr[i]*(s1*s2*(1-s3)+cs12-cs13-cs23)+(1-pr[i])*((1-c1)*(1-
c2)*c3+cc12-cc13-cc23);
   q[i,3]<-pr[i]*(s1*(1-s2)*s3-cs12+cs13-cs23)+(1-pr[i])*((1-c1)*c2*(1-c3)-
cc12+cc13-cc23);
   q[i,4]<-pr[i]*(s1*(1-s2)*(1-s3)-cs12-cs13+cs23)+(1-pr[i])*((1-c1)*c2*c3-cc12-
cc13+cc23);
   q[i,5]<-pr[i]*((1-s1)*s2*s3-cs12-cs13+cs23)+(1-pr[i])*(c1*(1-c2)*(1-c3)-cc12-
cc13+cc23);
   q[i,6]<-pr[i]*((1-s1)*s2*(1-s3)-cs12+cs13-cs23)+(1-pr[i])*(c1*(1-c2)*c3-
cc12+cc13-cc23);
   q[i,7]<-pr[i]*((1-s1)*(1-s2)*s3+cs12-cs13-cs23)+(1-pr[i])*(c1*c2*(1-
c3)+cc12-cc13-cc23);
   q[i,8]<-pr[i]*((1-s1)*(1-s2)*(1-s3)+cs12+cs13+cs23)+(1-
pr[i])*(c1*c2*c3+cc12+cc13+cc23);

############################################################
## Check and correct potential errors of probabilities exceeding (0,1) bounds
############################################################

checks[i,1]<-   s1*s2*s3+cs12+cs13+cs23;
```

Text S2: Bayesian latent class model code for three diagnostic tests (cont.)

```
  checks[i,2]<-   (1-c1)*(1-c2)*(1-c3)+cc12+cc13+cc23;
  checks[i,3]<-  s1*s2*(1-s3)+cs12-cs13-cs23;
  checks[i,4]<-  (1-c1)*(1-c2)*c3+cc12-cc13-cc23;
  checks[i,5]<-  s1*(1-s2)*s3-cs12+cs13-cs23;
  checks[i,6]<-  (1-c1)*c2*(1-c3)-cc12+cc13-cc23;
  checks[i,7]<-  s1*(1-s2)*(1-s3)-cs12-cs13+cs23;
  checks[i,8]<-  (1-c1)*c2*c3-cc12-cc13+cc23;
  checks[i,9]<-  (1-s1)*s2*s3-cs12-cs13+cs23;
  checks[i,10]<- c1*(1-c2)*(1-c3)-cc12-cc13+cc23;
  checks[i,11]<- (1-s1)*s2*(1-s3)-cs12+cs13-cs23;
  checks[i,12]<- c1*(1-c2)*c3-cc12+cc13-cc23;
  checks[i,13]<- (1-s1)*(1-s2)*s3+cs12-cs13-cs23;
  checks[i,14]<- c1*c2*(1-c3)+cc12-cc13-cc23;
  checks[i,15]<- (1-s1)*(1-s2)*(1-s3)+cs12+cs13+cs23;
  checks[i,16]<- c1*c2*c3+cc12+cc13+cc23;

  valid[i]<- step(1-q[i,1])*step(q[i,1])*
   step(1-q[i,2])*step(q[i,2])*
   step(1-q[i,3])*step(q[i,3])*
   step(1-q[i,4])*step(q[i,4])*
   step(1-q[i,5])*step(q[i,5])*
   step(1-q[i,6])*step(q[i,6])*
   step(1-q[i,7])*step(q[i,7])*
   step(1-q[i,8])*step(q[i,8])*
   step(1-checks[i,1])*step(checks[i,1])*
   step(1-checks[i,2])*step(checks[i,2])*
   step(1-checks[i,3])*step(checks[i,3])*
   step(1-checks[i,4])*step(checks[i,4])*
   step(1-checks[i,5])*step(checks[i,5])*
   step(1-checks[i,6])*step(checks[i,6])*
   step(1-checks[i,7])*step(checks[i,7])*
   step(1-checks[i,8])*step(checks[i,8])*
   step(1-checks[i,9])*step(checks[i,9])*
   step(1-checks[i,10])*step(checks[i,10])*
   step(1-checks[i,11])*step(checks[i,11])*
   step(1-checks[i,12])*step(checks[i,12])*
   step(1-checks[i,13])*step(checks[i,13])*
   step(1-checks[i,14])*step(checks[i,14])*
   step(1-checks[i,15])*step(checks[i,15])*
   step(1-checks[i,16])*step(checks[i,16]);


############################################################
## Contribution to the likelihood for each observation
############################################################

L[i]<- equals(valid[i],1)*(
    equals(m.short [i,1],1)*equals(m.short[i,2],1)*equals(m.short [i,3],1)*q[i,1]
    + equals(m.short [i,1],1)*equals(m.short[i,2],1)*equals(m.short [i,3],0)*q[i,2]
```

Text S2: Bayesian latent class model code for three diagnostic tests (cont.)

```
    + equals(m.short [i,1],1)*equals(m.short[i,2],0)*equals(m.short [i,3],1)*q[i,3]
    + equals(m.short [i,1],1)*equals(m.short[i,2],0)*equals(m.short [i,3],0)*q[i,4]
    + equals(m.short [i,1],0)*equals(m.short[i,2],1)*equals(m.short [i,3],1)*q[i,5]
    + equals(m.short [i,1],0)*equals(m.short[i,2],1)*equals(m.short [i,3],0)*q[i,6]
    + equals(m.short [i,1],0)*equals(m.short[i,2],0)*equals(m.short [i,3],1)*q[i,7]
    + equals(m.short [i,1],0)*equals(m.short[i,2],0)*equals(m.short [i,3],0)*q[i,8]
   ) +(1-equals(valid[i],1)) *(1e-14);

## When adding covariates to the model
logit(pr[i])<-intercept+slope*m.short[i,6];

##Without covariates:
pr[i]<-prc

############################################################
## Trick to ensure the probabilities are always less than 1
############################################################

p[i] <- L[i] / 1;## divided by a constant just to ensure all p's <1
     ones[i] ~ dbern(p[i]);
 }

############################################################
## Definition of model priors
############################################################
## Covariance terms
 covs12~dunif(-1,1);
 covs13~dunif(-1,1);
 covs23~dunif(-1,1);
 covc12<-0;
 covc13<-0;
 covc23<-0;

 prc~dbeta(37.9836,31.2593);      # Prevalence
 c1<-1;                           # SP necropsy-SCT
 c2~dbeta(1,1);                   # SP PCR
 c3~dbeta(1,1);                   # SP ELISA pab
 s1~dbeta(99.6983,6.1946);        # SE necropsy-SCT
 s2~dbeta(37.9836,31.2593);       # SE PCR
 s3~dbeta(1,1);                   # SE ELISA pab

 logL<-sum(log(p[1:N]));

## When adding covariates to the model
 #intercept~dnorm(0,0.001);
 #slope~dnorm(0,0.001);

 logL<-sum(log(p[1:N]));
  }
```

Text S3: Bayesian latent class model code for four diagnostic tests

Additional file 3. Bayesian latent-class model code for four diagnostic tests

```
############################################################
##Definition of the variables in the model
############################################################

var p[N], q[N,16], pr[N], L[N],checks[N,32];


#N <- observations (N=300 foxes)
# p <- individual samples
# q <- different combinations of test results
# pr <- prevalence
# s <- test sensitivities
# c <- test specificities
#  cs <- conditional dependency between tests sensitivities
#  cc <- conditional dependency between tests specificities
# m.short <- data set name


############################################################
## Modelling the different probabilities of combinations of tests results
############################################################

model {

  for(i in 1:N){

 q[i,1] <-pr[i]*(s1*s2*s3*s4+cs12+cs13+cs14+cs23+cs24+cs34) +(1-pr[i])*((1-
c1)*(1-c2)*(1-c3)*(1- c4)+cc12+ cc13+ cc14+ cc23+ cc24+cc34);
 q[i,2] <-pr[i]*(s1*s2*s3*(1-s4)+cs12+cs13-cs14+cs23-cs24-cs34) +(1-
pr[i])*((1-c1)*(1-c2)*(1-c3)*c4+cc12+cc13-cc14+cc23-cc24-cc34);
 q[i,3] <-pr[i]*(s1*s2*(1-s3)*s4+cs12-cs13+cs14-cs23+cs24-cs34) +(1-
pr[i])*((1-c1)*(1-c2)*c3*(1-c4)+cc12-cc13+cc14-cc23+cc24-cc34);
 q[i,4] <-pr[i]*(s1*s2*(1-s3)*(1-s4)+cs12-cs13-cs14-cs23-cs24+cs34) +(1-
pr[i])*((1-c1)*(1-c2)*c3*c4+cc12-cc13-cc14-cc23-cc24+cc34);
 q[i,5] <-pr[i]*(s1*(1-s2)*s3*s4-cs12+cs13+cs14-cs23-cs24+cs34) +(1-
pr[i])*((1-c1)*c2*(1-c3)*(1-c4)-cc12+cc13+cc14-cc23-cc24+cc34);
 q[i,6] <-pr[i]*(s1*(1-s2)*s3*(1-s4)-cs12+cs13-cs14-cs23+cs24-cs34) +(1-
pr[i])*((1-c1)*c2*(1-c3)*c4-cc12+cc13-cc14-cc23+cc24-cc34);
 q[i,7] <-pr[i]*(s1*(1-s2)*(1-s3)*s4-cs12-cs13+cs14+cs23-cs24-cs34) +(1-
pr[i])*((1-c1)*c2*c3*(1-c4)-cc12-cc13+cc14+cc23-cc24-cc34);
 q[i,8] <-pr[i]*(s1*(1-s2)*(1-s3)*(1-s4)-cs12-cs13-cs14+cs23+cs24+cs34) +(1-
pr[i])*((1-c1)*c2*c3*c4-cc12-cc13-cc14+cc23+cc24+cc34);
 q[i,9] <-pr[i]*((1-s1)*s2*s3*s4-cs12-cs13-cs14+cs23+cs24+cs34) +(1-
pr[i])*(c1*(1-c2)*(1-c3)*(1-c4)-cc12-cc13-cc14+cc23+cc24+cc34);
 q[i,10]<-pr[i]*((1-s1)*s2*s3*(1-s4)-cs12-cs13+cs14+cs23-cs24-cs34) +(1-
pr[i])*(c1*(1-c2)*(1-c3)*c4-cc12-cc13+cc14+cc23-cc24-cc34);
```

Text S3: Bayesian latent class model code for four diagnostic tests (cont.)

```
 q[i,11]<-pr[i]*((1-s1)*s2*(1-s3)*s4-cs12+cs13-cs14-cs23+cs24-cs34)+(1-
pr[i])*(c1*(1-c2)*(c3)*(1-c4)-cc12+cc13-cc14-cc23+cc24-cc34);
 q[i,12]<-pr[i]*((1-s1)*s2*(1-s3)*(1-s4)-cs12+cs13+cs14-cs23-cs24+cs34)+(1-
pr[i])*(c1*(1-c2)*c3*c4-cc12+cc13+cc14-cc23-cc24+cc34);
 q[i,13]<-pr[i]*((1-s1)*(1-s2)*s3*s4+cs12-cs13-cs14-cs23-cs24+cs34)+(1-
pr[i])*(c1*c2*(1-c3)*(1-c4)+cc12-cc13-cc14-cc23-cc24+cc34);
 q[i,14]<-pr[i]*((1-s1)*(1-s2)*s3*(1-s4)+cs12-cs13+cs14-cs23+cs24-cs34)+(1-
pr[i])*(c1*c2*(1-c3)*c4+cc12-cc13+cc14-cc23+cc24-cc34);
 q[i,15]<-pr[i]*((1-s1)*(1-s2)*(1-s3)*s4+cs12+cs13-cs14+cs23-cs24-cs34)+(1-
pr[i])*(c1*c2*c3*(1-c4)+cc12+cc13-cc14+cc23-cc24-cc34);
 q[i,16]<-pr[i]*((1-s1)*(1-s2)*(1-s3)*(1-
s4)+cs12+cs13+cs14+cs23+cs24+cs34)+(1-
pr[i])*(c1*c2*c3*c4+cc12+cc13+cc14+cc23+cc24+cc34);

############################################################
## Check and correct potential errors of probabilities exceeding (0,1) bounds
############################################################

   checks[i,1]<-  s1*s2*s3*s4+cs12+cs13+cs14+cs23+cs24+cs34;
  checks[i,2]<-  (1-c1)*(1-c2)*(1-c3)*(1-c4) +cc12+ cc13+ cc14+ cc23
+cc24+cc34;
  checks[i,3]<-  s1*s2*s3*(1-s4)+cs12+cs13-cs14+cs23-cs24-cs34;
  checks[i,4]<-  (1-c1)*(1-c2)*(1-c3)*c4+cc12+cc13-cc14+cc23-cc24-cc34;
  checks[i,5]<-  s1*s2*(1-s3)*s4+cs12-cs13+cs14-cs23+cs24-cs34
  checks[i,6]<-  (1-c1)*(1-c2)*c3*(1-c4)+cc12-cc13+cc14-cc23+cc24-cc34;
  checks[i,7]<-  s1*s2*(1-s3)*(1-s4)+cs12-cs13-cs14-cs23-cs24 + cs34;
  checks[i,8]<-  (1-c1)*(1-c2)*c3*c4+cc12-cc13-cc14-cc23-cc24+ cc34;
  checks[i,9]<-  s1*(1-s2)*s3*s4-cs12+cs13+cs14-cs23-cs24+cs34;
  checks[i,10]<- (1-c1)*c2*(1-c3)*(1-c4)-cc12+cc13+cc14-cc23-cc24+cc34;
  checks[i,11]<- s1*(1-s2)*s3*(1-s4)-cs12+cs13-cs14-cs23+cs24-cs34;
  checks[i,12]<- (1-c1)*c2*(1-c3)*c4-cc12+cc13-cc14-cc23+cc24-cc34;
  checks[i,13]<- s1*(1-s2)*s3*(1-s4)-cs12+cs13-cs14-cs23+cs24-cs34;
  checks[i,14]<- (1-c1)*c2*c3*(1-c4)-cc12-cc13+cc14+cc23-cc24-cc34;
  checks[i,15]<- s1*(1-s2)*(1-s3)*(1-s4)-cs12-cs13-cs14+cs23+ cs24+cs34;
  checks[i,16]<- (1-c1)*c2*c3*c4-cc12-cc13-cc14+cc23+ cc24+ cc34;
  checks[i,17]<- (1-s1)*s2*s3*s4-cs12-cs13-cs14+cs23+ cs24+ cs34;
  checks[i,18]<- c1*(1-c2)*(1-c3)*(1-c4)-cc12-cc13-cc14+cc23+ cc24+cc34;
  checks[i,19]<- (1-s1)*s2*s3*(1-s4)-cs12-cs13+cs14+cs23-cs24-cs34;
  checks[i,20]<- c1*(1-c2)*(1-c3)*c4-cc12-cc13+cc14+cc23-cc24-cc34;
  checks[i,21]<- (1-s1)*s2*(1-s3)*s4-cs12+cs13-cs14-cs23+cs24-cs34;
  checks[i,22]<- c1*(1-c2)*(c3)*(1-c4)-cc12+cc13-cc14-cc23+cc24-cc34;
  checks[i,23]<- (1-s1)*s2*(1-s3)*(1-s4)-cs12+cs13+cs14-cs23-cs24+cs34;
  checks[i,24]<- c1*(1-c2)*c3*c4-cc12+cc13+cc14-cc23-cc24+cc34;
  checks[i,25]<- (1-s1)*(1-s2)*s3*s4+cs12-cs13-cs14-cs23-cs24+cs34;
  checks[i,26]<- c1*c2*(1-c3)*(1-c4)+cc12-cc13-cc14-cc23-cc24+cc34;
  checks[i,27]<- (1-s1)*(1-s2)*s3*(1-s4)+cs12-cs13+cs14-cs23+ cs24-cs34;
  checks[i,28]<- c1*c2*(1-c3)*c4+cc12-cc13+cc14-cc23+cc24-cc34;
  checks[i,29]<- (1-s1)*(1-s2)*(1-s3)*s4+cs12+cs13-cs14+cs23-cs24-cs34;
  checks[i,30]<- c1*c2*c3*(1-c4)+cc12+cc13-cc14+cc23-cc24-cc34;
```

Text S3: Bayesian latent class model code for four diagnostic tests (cont.)

```
  checks[i,31]<- (1-s1)*(1-s2)*(1-s3)*(1-s4)+cs12+cs13+cs14+ cs23+
cs24+cs34;
  checks[i,32]<- c1*c2*c3*c4+cc12+cc13+cc14+cc23+cc24+cc34;


 valid[i]<- step(1-q[i,1])*step(q[i,1])*
        step(1-q[i,2])*step(q[i,2])*
        step(1-q[i,3])*step(q[i,3])*
        step(1-q[i,4])*step(q[i,4])*
        step(1-q[i,5])*step(q[i,5])*
        step(1-q[i,6])*step(q[i,6])*
        step(1-q[i,7])*step(q[i,7])*
        step(1-q[i,8])*step(q[i,8])*
        step(1-q[i,9])*step(q[i,9])*
        step(1-q[i,10])*step(q[i,10])*
        step(1-q[i,11])*step(q[i,11])*
        step(1-q[i,12])*step(q[i,12])*
        step(1-q[i,13])*step(q[i,13])*
        step(1-q[i,14])*step(q[i,14])*
        step(1-q[i,15])*step(q[i,15])*
        step(1-q[i,16])*step(q[i,16])*
        step(1-checks[i,1])*step(checks[i,1])*
        step(1-checks[i,2])*step(checks[i,2])*
        step(1-checks[i,3])*step(checks[i,3])*
        step(1-checks[i,4])*step(checks[i,4])*
        step(1-checks[i,5])*step(checks[i,5])*
        step(1-checks[i,6])*step(checks[i,6])*
        step(1-checks[i,7])*step(checks[i,7])*
        step(1-checks[i,8])*step(checks[i,8])*
        step(1-checks[i,9])*step(checks[i,9])*
        step(1-checks[i,10])*step(checks[i,10])*
        step(1-checks[i,11])*step(checks[i,11])*
        step(1-checks[i,12])*step(checks[i,12])*
        step(1-checks[i,13])*step(checks[i,13])*
        step(1-checks[i,14])*step(checks[i,14])*
        step(1-checks[i,15])*step(checks[i,15])*
        step(1-checks[i,16])*step(checks[i,16])*
        step(1-checks[i,17])*step(checks[i,17])*
        step(1-checks[i,18])*step(checks[i,18])*
        step(1-checks[i,19])*step(checks[i,19])*
        step(1-checks[i,20])*step(checks[i,20])*
        step(1-checks[i,21])*step(checks[i,21])*
        step(1-checks[i,22])*step(checks[i,22])*
        step(1-checks[i,23])*step(checks[i,23])*
        step(1-checks[i,24])*step(checks[i,24])*
        step(1-checks[i,25])*step(checks[i,25])*
        step(1-checks[i,26])*step(checks[i,26])*
        step(1-checks[i,27])*step(checks[i,27])*
        step(1-checks[i,28])*step(checks[i,28])*
```

Text S3: Bayesian latent class model code for four diagnostic tests (cont.)

```
        step(1-checks[i,29])*step(checks[i,29])*
        step(1-checks[i,30])*step(checks[i,30])*
        step(1-checks[i,31])*step(checks[i,31])*
        step(1-checks[i,32])*step(checks[i,32]);



#########################################################
## Contribution to the likelihood for each observation
#########################################################

  L[i]<- equals(valid[i],1)*(

equals(m.short[i,1],1)*equals(m.short[i,2],1)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],1)*q[i,1]
      +
equals(m.short[i,1],1)*equals(m.short[i,2],1)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],0)*q[i,2]
      +
equals(m.short[i,1],1)*equals(m.short[i,2],1)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],1)*q[i,3]
      +
equals(m.short[i,1],1)*equals(m.short[i,2],1)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],0)*q[i,4]
      +
equals(m.short[i,1],1)*equals(m.short[i,2],0)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],1)*q[i,5]
      +
equals(m.short[i,1],1)*equals(m.short[i,2],0)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],0)*q[i,6]
      +
equals(m.short[i,1],1)*equals(m.short[i,2],0)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],1)*q[i,7]
      +
equals(m.short[i,1],1)*equals(m.short[i,2],0)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],0)*q[i,8]
      +
equals(m.short[i,1],0)*equals(m.short[i,2],1)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],1)*q[i,9]
      +
equals(m.short[i,1],0)*equals(m.short[i,2],1)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],0)*q[i,10]
      +
equals(m.short[i,1],0)*equals(m.short[i,2],1)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],1)*q[i,11]
```

Text S3: Bayesian latent class model code for four diagnostic tests (cont.)

```
      +
equals(m.short[i,1],0)*equals(m.short[i,2],1)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],0)*q[i,12]
      +
equals(m.short[i,1],0)*equals(m.short[i,2],0)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],1)*q[i,13]
      +
equals(m.short[i,1],0)*equals(m.short[i,2],0)*equals(m.short[i,3],1)*equals(m.sh
ort[i,4],0)*q[i,14]
      +
equals(m.short[i,1],0)*equals(m.short[i,2],0)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],1)*q[i,15]
      +
equals(m.short[i,1],0)*equals(m.short[i,2],0)*equals(m.short[i,3],0)*equals(m.sh
ort[i,4],0)*q[i,16]
     ) +(1-equals(valid[i],1)) *(1e-14);


## When adding covariates to the model
logit(pr[i])<-intercept+slope*m.short[i,5];

##Without covariates:
pr[i]<-prc

############################################################
## Trick to ensure the probabilities are always less than 1
############################################################

p[i] <- L[i] / 1;## divided by a constant just to ensure all p's <1
    ones[i] ~ dbern(p[i]);
 }

############################################################
## Definition of model priors
############################################################

 prc~dbeta(37.9836,31.2593);       # Prev
 c1<-1;                            # Specificity necropsy-SCT fixed
 c2~dbeta(36.7028,2.8791);         # Specificity PCR
 c3~dbeta(1,1);                    # Specificity pa-ELISA
 c4~dbeta(1,1);                    # Specificity ma-ELISA
 s1~dbeta(99.6983,6.1946);         # Sensitivity necropsy-SCT
 s2~dbeta(37.9836,31.2593);        # Sensitivity PCR
 s3~dbeta(1,1);                    # Sensitivity pa-ELISA
 s4~dbeta(1,1);                    # Sensitivity ma-ELISA

## Covariance terms
 cs12~dunif(-1,1);
 cs13~dunif(-1,1);
```

Text S3: Bayesian latent class model code for four diagnostic tests (cont.)

```
 cs14~dunif(-1,1);
 cs23~dunif(-1,1);
 cs24~dunif(-1,1);
 cs34~dunif(-1,1);
 cc12<-0;
 cc13<-0;
 cc14<-0;
 cc23<-0;
 cc24<-0;
 cc34<-0;

## When adding covariates to the model
 #intercept~dnorm(0,0.001);
 #slope~dnorm(0,0.001);

 logL<-sum(log(p[1:N]));


  }
```

Text S4: Description of the prior information used in the latent class models for three diagnostic tests

Additional file 4. Description of the prior information used in the latent class models for three diagnostic tests

Parameters		**Distribution**
		dunif()
Covariances	Sensitivities	(-1,1)
Covariances	Specificity	- Fixed to 0
		dbeta(a,b)
Necropsy-SCT	Sensitivity	(99.6983,6.1946)[a]
	Specificity	- Fixed to 1
Egg-PCR	Sensitivity	(37.9836,31.2593)[b]
	Specificity	(1,1)
pAb-ELISA	Sensitivity	(1,1)
	Specificity	(1,1)
Prevalence		(37.9836,31.2593)[c]

All informative priors were obtained by using betabuster (http://cadms.ucdavis.edu/diagnostictests/betabuster.html), entering the following
[a] Being 95% sure, that the sensitivity of necropsy-SCT is larger than 0.9 with a mode at 0.95
[b] Being 95% sure, that the sensitivity of egg-PCR is larger than 0.45 with a mode at 0.55
[c] Being 95% sure, that the prevalence is larger than 0.45 with a mode at 0.55

Text S5: Description of the prior information used in the latent class models for four diagnostic tests

Additional file 5. Description of the prior information used in the latent class models for four diagnostic tests

Parameters		**Distribution**
		dunif()
Covariances	Sensitivities	(-1,1)
Covariances	Specificity	- Fixed to 0
		dbeta(a,b)
Necropsy-SCT	Sensitivity	(99.6983,6.1946)[a]
	Specificity	- Fixed to 1
Egg-PCR	Sensitivity	(37.9836,31.2593)[b]
	Specificity	(36.7028,2.8791)[c]
pAb-ELISA	Sensitivity	(1,1)
	Specificity	(1,1)
mAb-ELISA	Sensitivity	(1,1)
	Specificity	(1,1)
Prevalence		(37.9836,31.2593)[d]

All informative priors were obtained by using betabuster (http://cadms.ucdavis.edu/diagnostictests/betabuster.html), entering the following
[a] Being 95% sure, that the sensitivity of necropsy-SCT is larger than 0.9 with a mode at 0.95
[b] Being 95% sure, that the sensitivity of egg-PCR is larger than 0.45 with a mode at 0.55
[c] Being 95% sure, that the specificity of egg-PCR is larger than 0.85 with a mode at 0.95
[d] Being 95% sure, that the prevalence is larger than 0.45 with a mode at 0.55

Text S6: Supplementary sensitivity analysis of PCR

Supplementary sensitivity analysis (PCR)

July 2017

Sensitivity analysis to assess the robustness of the Bayesian latent class analysis:

We used informative priors, based on literature, for prevalence, sensitivity of necropsy, sensitivity and specificity of PCR. Values for the specific beta distributions were obtained with the program betabuster http://cadms.ucdavis.edu/diagnostictests/betabuster.html.

- 1) Constant informative prior on prevalence: dbeta(37.9836, 31.2593) obtained by betabuster "being 95% sure, that the prevalence is larger than 0.45 with a mode at 0.55".
- 2) Constant informative prior on sensitivity of necropsy: dbeta(99.6983,6.1946) obtained by betabuster "being 95% sure, that the sensitivity of necropsy is larger than 0.9 with a mode at 0.95".

We varied the informative prior for the sensitivity of the PCR systematically from assuming that the sensitivity is larger than 0.9, 0.8 and so on until 0.1, with a respective mode of 0.95, 0.85 and so on until 0.25. With this approach we obtained a number of informative priors, ranging from strong priors with a small variance (steep curve) or high precision, e.g. "greater than 60 % with a mode at 65%" to rather uninformative priors e.g. "greater than 10% and a mode at 95%" (flat curve). The latter one is close to the independence model with priors dbeta(1,1). Furthermore with this approach we also obtained a number of priors which are - potentially- in conflict with our data, e.g. we assume that the sensitivity is not close to 95% or 25%.

The idea was (in line with Jim Albert in "Bayesian computation with R", 2nd ed., 2009 Springer on page 45 "[. . .] where different priors are possible, it is desirable that inferences from the posterior not to be dependent from the exact functional form of the prior. A Bayesian analysis is said to be robust to the choice of prior if the inference is insensitive to different priors that match the user's belief."

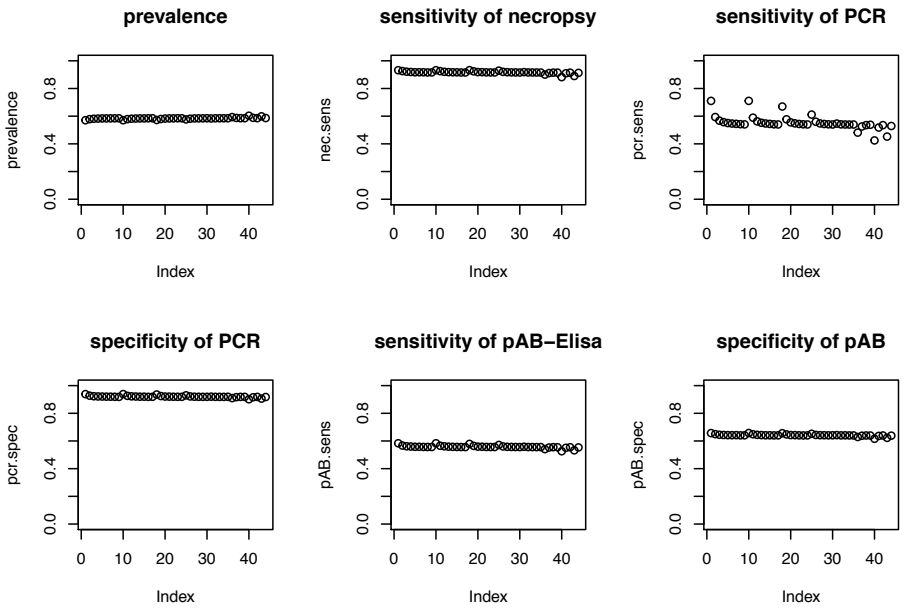
We deliberately chose a number of informative priors which match our prior beliefs by successively allowing a wider range of the prior to assess the prior's influence on the posterior density distribution. Although Bayesian analysis allows for incorporating "subjective" prior information (which might be useful in some cases) in our analysis we wanted the data (or the likelihood thereof) to be the main drivers for the posteriors. Thus we expected to see - for priors in disagreement with the data - a different posterior distribution compared to the posteriors of our model in the main paper and this difference being more pronounced with stronger priors.

When looking at the 44 models run with different informative priors consecutively, a clear pattern for the sensitivity of PCR - which we varied systematically - becomes clear. A similar pattern although considerably less pronounced is also present in the five other parameters of interest. For the posterior sensitivities of PCR, the models 1,10,18,25 are clearly above and the models 36, 40 and 45 are clearly below the estimates when informative priors in agreement with existing knowledge. All these models have in common that relatively strong priors (steep curve) due to assuming that "we are 95% sure that the sensitivity of PCR is larger than 0.9 with a mode of 0.95", ". . . larger than 0.8 with a mode of 0.85" and so on in steps of 0.1 until ". . . larger than 0.2 with a mode of 0.25". The sum of a and b for this model is also approx. 50% of the sample size, indicating also the high, presumably too high prior strength for this data set.

In the following first, the means of the posteriors for the prevalence and each of the test accuracies estimated in the three-test model in the 44 models are shown. Then, for purpose of illustration, the densities for the first model (out of 44), including the prior as well as the posterior densities with informative priors and covariances as well as under independence assumption.

In a similar way - we also run sensitivity analyses for varying the sensitivity of necropsy and prevalence (not shown) - we believe that our results are robust, i.e. not influenced by the informative priors.

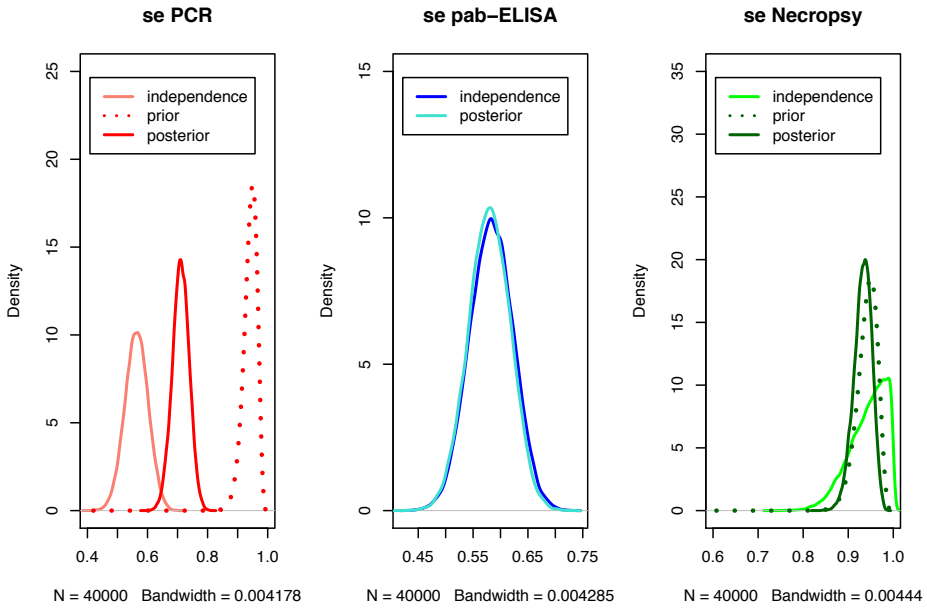
Text S6: Supplementary sensitivity analysis of PCR (cont.)



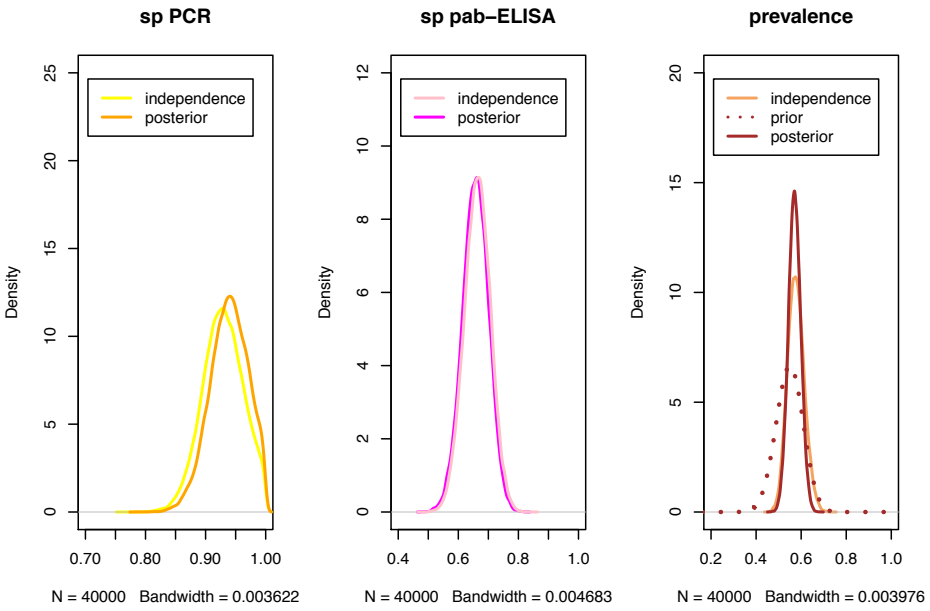
Text S6: Supplementary sensitivity analysis of PCR (cont.)

1) density plots for prior for sensitivity of PCR beta(99.6983, 6.1946)

meaning: 95 % sure, that se of PCR is greater than 0.9 with a mode at 0.95.



Text S6: Supplementary sensitivity analysis of PCR (cont.)



Text S6: Supplementary sensitivity analysis of PCR (cont.)

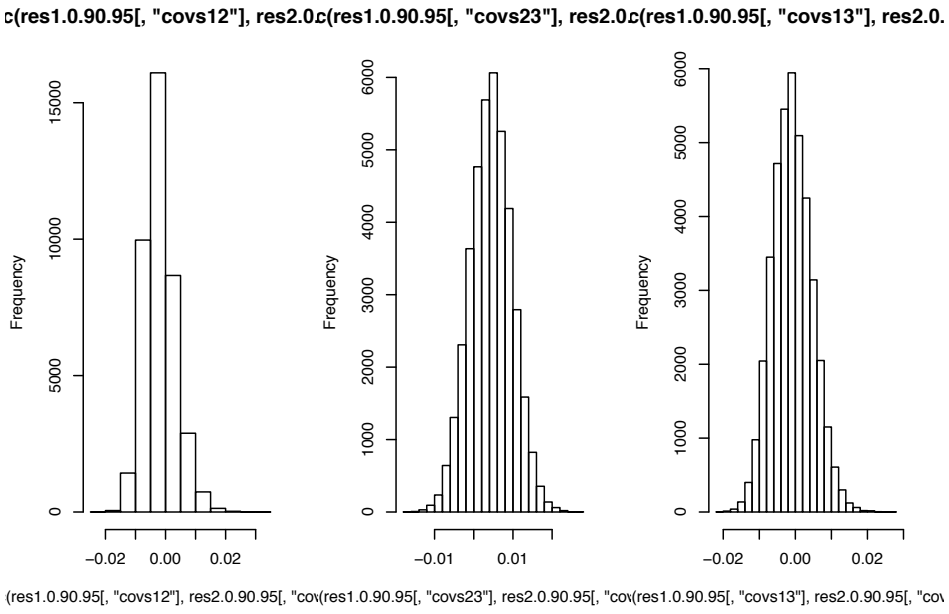


Figure S7: Polyclonal ELISA ROC curves produced using the classical and the Bayesian approach (4 tests)

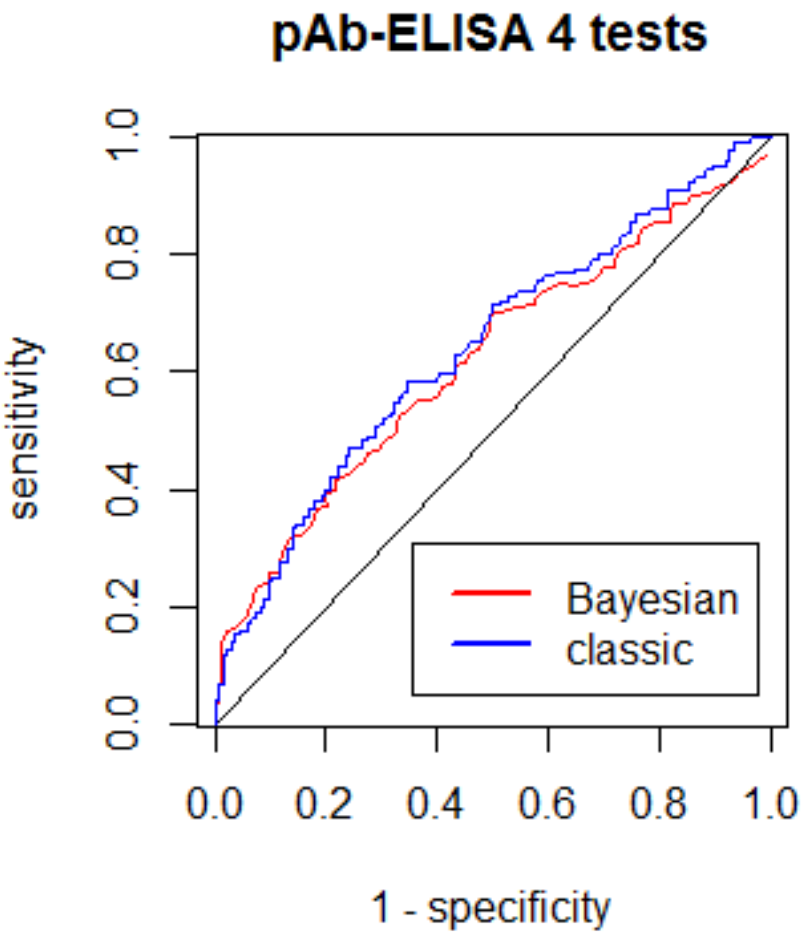
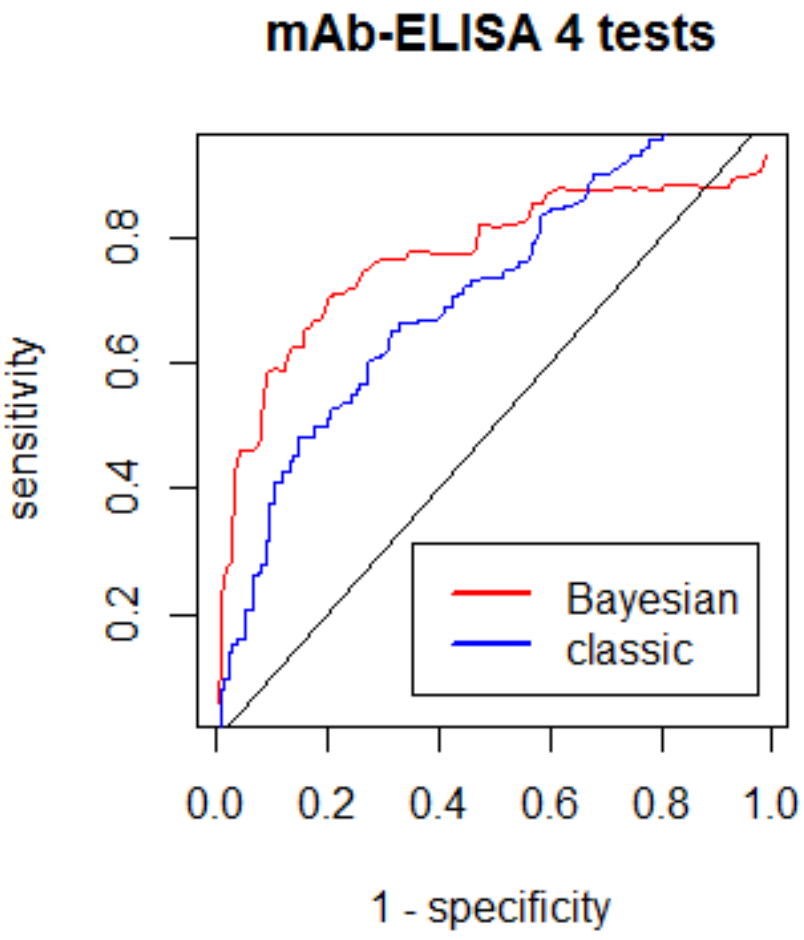


Figure S8: Monoclonal ELISA ROC curves produced using the classical and the Bayesian approach (4 tests)



Text S9: Resulting covariances between sensitivities of the 3 and 4 diagnostic tests models

Additional file 9. Resulting covariances between sensitivities of the 3 and 4 test model

Covariance between sensitivities of	3 test model	4 test model
SCT and PCR	0.0086	0.0021
SCT and pAb-Elisa	0.0100	0.0019
PCR and pAb-Elisa	0.0094	0.0042
SCT and mAb-Elisa	NA[c]	0.0033
PCR and mAb-Elisa	NA[c]	0.0043
pAb-Elisa and mAb-Elisa	NA[c]	0.0077

NA[c]: not applicable

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EDUCATION

09.2008-07.2009 Royal Veterinary College and London School of Hygiene and Tropical Medicine, London, United Kingdom:
Masters Degree in Veterinary Epidemiology
Thesis: *Qualitative Risk Assessment and efficiency estimation of pre-harvest Salmonella control interventions in the Catalonian pork chain (Spain)*

09.1997-07.2004 Cardenal Herrera-CEU Veterinary School, Valencia, Spain and Facoltà di Medicina Veterinaria, Bologna, Italy:
Bachelor in Veterinary Medicine (BVetMed)

PROFESSIONAL EXPERIENCE

07.2011-08.2015 Epidemiology Section, Vetsuisse Faculty, University of Zurich, Switzerland: **Research Assistant (PhD studies)**
Topic: *Mathematical modelling of parasitic diseases*
- Data collection and laboratory diagnostic testing
- Statistical analysis, modelling and software programming
- Writing and publication of scientific papers
- Conference and posters presentations of research findings
- Teaching assistance for undergraduate lectures

10.2009-05.2011 Epidemiology and Public Health Group, Royal Veterinary College, London, United Kingdom:
Research Assistant
Projects: *Cost-benefit analysis for setting a target for Salmonella reduction in slaughter and breeding pigs* (SANCO/2008/E2/036 and 056) and *Improving consumer protection against zoonotic diseases in Albania* (EUROPEAID/128304/C/SER/AL)
- Socio-economic analysis of animal diseases and policy-making
- Data collection on livestock diseases at country-level
- Questionnaire design and interview of stakeholders

07.2006-03.2009 Hall Mark Hygiene Ltd., England and Wales, United Kingdom:
Official Veterinarian (OV)
- Ante-Mortem and Post-Mortem inspection of livestock
- Meat inspection and enforcement of Food Regulations
- Enforcement of Animal Welfare and Transport Regulations
- Audit of good hygiene practices and HACCP-based procedures
- Team management and administrative oversight

01.2006-02.2006	Mediterranean Hospital for Small Animals, Valencia, Spain: **Veterinary Surgeon** - Prevention, diagnosis and treatment of animal illnesses
02.2005-09.2005	Eville & Jones Ltd., Shrewsbury, United Kingdom: **Meat Hygiene Inspector** - Meat inspection of animal carcasses - Monitoring and recording of pathological lesions

ADDITIONAL RELEVANT EDUCATION AND TRAINING

02.2012	Wellcome Trust Advanced Courses, Cambridge, United Kingdom: **Mathematical Models for Infectious Disease Dynamics Certificate**
03.2007	Department for Environment, Food and Rural Affairs, United Kingdom: **Local Veterinary Inspector Exports Certificate**
06.2006	School of Veterinary Medicine, University of Glasgow, United Kingdom: **Official Veterinarian Accreditation**
08.2004	Public Health Department, Valencia, Spain: **Public Health Internship Certificate**
09.2003-07.2004	Facoltà di Medicina Veterinaria, Bologna, Italy: **Food Hygiene Training (ERASMUS scholarship)**

LANGUAGE SKILLS

Spanish	Mother tongue	Catalan	Intermediate (B1)
English	Advanced (C1)	German	Basic (A2)
Italian	Intermediate (B1)	French	Basic (A1)

COMPUTER SKILLS

Operating systems	Windows, OSX
Microsoft Office	Excel, Outlook, Power Point, Word
Statistical software	R, SPSS, Stata 10, JAGS
Others	Spatial Analysis (QGIS, ArcGIS), EndNote, Mendeley

PUBLICATIONS

Otero-Abad B, Torgerson PR (2013) A Systematic Review of the Epidemiology of Echinococcosis in Domestic and Wild Animals. PLoS Negl Trop Dis 7(6): e2249. doi:10.1371/journal.pntd.0002249

Lewis FI, Otero-Abad B, Hegglin D, Deplazes P, Torgerson PR (2014) Dynamics of the Force of Infection: Insights from *Echinococcus multilocularis* Infection in Foxes. PLoS Negl Trop Dis 8(3): e2731. doi:10.1371/journal.pntd.0002731

Otero-Abad B, Rüegg SR, Hegglin D, Deplazes P, Torgerson PR (2017). Mathematical modelling of *Echinococcus multilocularis* abundance in foxes in Zurich, Switzerland. Parasit. Vectors, 10:21. doi:10.1186/s13071-016-1951-1